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1 **Essential oil of the Malagasy grass *Elionurus tristis* Hack. contains several**  
2 **undescribed sesquiterpenoids.**

3

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17

18 **Abstract**

19 Essential oils (EOs) obtained from aerial parts and roots of *Elionurus tristis* were investigated  
20 by GC, GC-MS, pc-GC and NMR. Both aerial parts and roots EOs contained common  
21 molecules such as  $\alpha$ -pinene, camphene, *trans*- $\alpha$ -bergamotene and calarene. Moreover, we  
22 identified several unusual sesquiterpenes and four undescribed compounds, 7-*epi*-khusian-2-  
23 ol, 4,8-di-*epi*-acorone, 2-*epi*-ziza-5-en-2-ol and antsorenone. The last one exhibits an

24 undescribed natural sesquiterpene skeleton. All undescribed compounds were isolated and  
25 fully characterized by MS, 1D- and 2D-NMR. Furthermore, the formation pathway of the  
26 Antsorane skeleton is discussed.

27

28 **Keywords:** *Elionurus tristis* Hack., Poaceae, antsorane skeleton, antsorenone, 2-*epi*-ziza-5-  
29 en-2-ol, 7-*epi*-khusian-2-ol, 4,8-di-*epi*-acorone, NMR, pc-GC.

30

## 31 **1. Introduction**

32 The members of the genus *Elionurus* (Poaceae) is mainly found in tropical and  
33 subtropical regions of America, Africa, and Australia (Renvoize, 1978). While previously the  
34 generic name has also been spelt *Elyonurus*, the spelling *Elionurus* is that now accepted by  
35 the Plant List (The Plant List, 2013). This genus contains less than 20 species, from which a  
36 very limited number have already been investigated for their essential oils and/or extracts, *E.*  
37 *viridulus* Hack. ex Stuck. synonym of *Elionurus muticus* (Spreng.) Kuntze (Chagonda et al.  
38 2000, 2012; Hefendehl and Fonseca, 1976; Scramin et al., 2000), *E. elegans* Kunth (Mevy et  
39 al., 2002) and *E. hensii* K.Schum. (Silou et al., 2006; Yang et al., 2013).

40 *Elionurus tristis* Hack. (Poaceae) commonly named “Antsoro” by the Malagasy is an  
41 aromatic grass endemic to the highlands of Madagascar, which has long been used by the  
42 local population as a folk medicine against colic but also as an antipyretic (Chazan, 1969).  
43 Although the very first study dealing with the essential oil of *E. tristis* was published more  
44 than forty years ago, the scientific literature related to *E. tristis* remains very scarce, and the  
45 actual knowledge of its volatile constituents rather unexplored. Nonetheless, the presence of  
46 an unusual sesquiterpenic ketone named antsorenone was pointed out by Chazan in 1969 but  
47 its structure could not be elucidated (Chazan, 1969). Recently another research group  
48 investigated the essential oil of *E. tristis* and reported a composition dominated by calarene  
49 (18.4%), neoclovene (15.8%), and nootkatone (10.4%) (Yedomon et al., 2017).

50 As a part of our three decades-long investigation of EOs from Madagascar, we had the  
51 opportunity to reinvestigate the volatile constituents of *E. tristis* growing wild in the central  
52 regions of the island. Herein, we report the detailed analysis of the chemical composition of  
53 EOs obtained from both the aerial parts and the roots of *E. tristis*. This analytical study was  
54 mainly conducted by means of gas chromatographic techniques (GC, GC-MS, pc-GC) and

55 NMR as an identification tool for the study of complex mixtures, but also for structure  
56 elucidation. Several undescribed sesquiterpenoids were isolated from *E. tristis* EOs and their  
57 structures were unambiguously characterized by NMR. Evident stereochemical relationships  
58 observed between these compounds led us establishing a possible biogenetic pathway for the  
59 production of the volatile constituents in the plant.

60

## 61 **2. Results and discussion**

### 62 *2.1. Analysis of E. tristis essential oils by GC(RI), GC-MS and <sup>13</sup>C NMR*

63 Essential oils of *E. tristis* were extracted from both the aerial parts and its roots as  
64 soon as the plant was harvested. EOs were subsequently analyzed by GC(RI) on both apolar  
65 (DB-1) and polar (DB-Wax) stationary phases using linear temperature programs for a  
66 reliable calculation of retention indices (RI). Constituents were identified upon combination  
67 of RI, MS, and <sup>13</sup>C NMR. The <sup>13</sup>C NMR based computerized methodology has been  
68 developed in our laboratory and allows the direct identification of constituents in mixtures  
69 such as essential oils or their fractions by comparison of their <sup>13</sup>C NMR values with those of  
70 compounds present in our in-house libraries. Each compound is identified by taking into  
71 account three parameters directly available from the software: (i) the number of carbon  
72 observed compared to what is expected, (ii) the difference between the chemical shift of each  
73 signal in the mixture and those from reference spectral data ( $\Delta\delta$ ), and (iii) the number of  
74 overlapped signals of carbons belonging to two components that fortuitously possess the same  
75 chemical shift (Ouattara et al., 2014; Tomi et al., 1995).

76 The major constituents identified in both the aerial parts and the roots EOs were  $\alpha$ -  
77 pinene (1.7; 7.3% respectively), camphene (4.0; 13.6%), *trans*- $\alpha$ -bergamotene (7.0; 3.3%) and  
78 calarene (2.1; 12.7%) Table 1). Interestingly, we detected the presence of ten undetermined

79 compounds (by GC(RI) and GC-MS) in appreciable amounts in EOs obtained from aerial  
80 parts (AP) and roots (R): i) compound **38** accounting for: 1.6% (AP), 2.2% (R); ii) **50**: 4.0%,  
81 6.3%; iii) **67**: 1.0%, 1.3%; iv) **69**: 5.1%, 1.8%; v) **71**: 17.6%, 7.2%; vi) **72**: 3.5%, 1.5%; vii)  
82 **75**: 0.6%, 3.1%; viii) **76**: 1.3%, 6.4%; ix) **77**: 15.4%, 5.6%; x) **78**: 3.1%, 0.2% (Fig. 1, 2 and  
83 S2).

## 84 2.2. *Identification of undetermined compounds*

85

86 In the process of analyzing the chemical composition of both the essential oils and  
87 their fractions, we noticed that four compounds named here **38**, **71**, **75**, and **76** remained  
88 undetermined, providing very unsatisfactory matching with commercial or in-house MS  
89 libraries. These four compounds could however be identified by applying our in-house  $^{13}\text{C}$   
90 NMR computerized methodology. Moreover, two rather uncommon molecules which were  
91 absent from our  $^{13}\text{C}$  NMR libraries were also identified after several fractionation steps, and  
92 their spectral characteristics could be reliably compared to literature data (compounds **50** and  
93 **69**) (see Experimental).

94

95 Compound **76** was enriched up to 56.9% in subfraction  $\text{F}_{\text{R}5-2}$ , and its  $^{13}\text{C}$  NMR values  
96 perfectly matched those previously reported for 1(10)-aristolen-2-one (Rücker et al., 1984)  
97 (Fig. 2 and S2, Table S1). Compound **75** was detected in several fractions and could be  
98 identified as aristolone by  $^{13}\text{C}$  NMR. **75** was further purified by means of repeated column  
99 chromatography (CC) on silica gel until 82.5% purity. Its full 2D-NMR data permitted to  
100 correct wrong  $^{13}\text{C}$  assignments reported by Su et al. (2009), mainly concerning methyl-14 and  
101 methyl-15, and the two methylenes C-2 and C-3 (Fig. 2 and S2, Table S4). Compound **71** was  
102 purified up to 89.9% purity (in  $\text{F}_{\text{AP}3}$ ) allowing the accurate assignment of all  $^1\text{H}$  and  $^{13}\text{C}$

103 signals through 2D-NMR sequences (Fig. 2 and S2, Table S3). This compound was  
104 unambiguously identified as acorenone B by its  $^{13}\text{C}$  NMR values, perfectly matching those  
105 reported by Kutschan et al. (1977).

106 RIs and MS spectrum of compound **38** were nearly identical to those of cyclosativene.  
107 However, its  $^{13}\text{C}$  NMR data showed significant differences with those of cyclosativene (for  
108 instance, C-2 was deshielded by +3.74 ppm and in contrast, C-6, C-8 and C-11 were shielded  
109 by -5.39, -4.26 and -3.51 ppm), mostly related to the presence of specific steric  $\gamma$ -effects,  
110 indicative of its stereoisomer, cyclocopacamphene, previously described by Garcia et al.  
111 (2017) (Fig. 2 and S2, Table S1).

112 Similarly, compound **50** showed similar RIs and MS data to those of prezizaene. However, its  
113  $^{13}\text{C}$  NMR chemical shifts differed from those of prezizaene, due to the presence of specific  
114 steric  $\gamma$ -effects (C-5 was deshielded by +4.82 ppm whereas, C-10 and C-12 were respectively  
115 shielded by -11.86 and -5.63 ppm). This led us to identify **50** as 2-*epi*-prezizaene which was  
116 previously described by Weyerstahl et al. (2000) (Fig. 2 and S2, Table S1).

117 Compound **69** (RI values 1640/2176 on both apolar and polar column) remained unknown  
118 from both our MS and  $^{13}\text{C}$  NMR libraries. Compound **69** was firstly enriched by CC up to  
119 18.8% in fraction  $F_{\text{AP}4}$  from the AP oil. This fraction was then submitted to preparative  
120 capillary-gas chromatography (pc-GC), yielding compound **69** with 94.6% purity. GC-MS  
121 analysis revealed a molecular ion at  $m/z$  222. Combination of  $^{13}\text{C}$  and DEPT spectra allowed  
122 to identify 15 carbon signals distributed as 3 C, 3 CH, 5  $\text{CH}_2$  and 4  $\text{CH}_3$ . The detailed  
123 examination of HSQC, HMBC and COSY spectra permitted to establish the basic prezizaene  
124 structure of the molecule **69**. Moreover, similarities of  $^{13}\text{C}$  NMR signals with those of  
125 compound **50** hydrocarbon revealed that **69** could be an oxygenated analogue of 2-*epi*-  
126 prezizaene. The quaternary carbon at 76.3 ppm indicated the presence of a tertiary alcohol  
127 group located on C-7. Furthermore, the stereochemistry of C-7 was deduced from the NOESY

128 correlation between H-9 and H-15. Compound **69** was finally identified as 2-*epi*-prezizaan-7-  
129 ol which was previously reported by Weyerstahl et al. (2000) as constituent of vetiver oil. The  
130 full 2D-NMR eventually allowed us to correct the wrong assignment made by Weyerstahl et  
131 al. (2000) for methyl groups 13 and 15 (Fig. 2 and S2, Table S2).

132

### 133 2.3. Identification of undescribed compounds

#### 134 2.3.1. Structure Elucidation of 7-*epi*-Khusian-2-ol (**72**) and 4,8-di-*epi*-Acorone (**78**)

135 The unidentified compounds **72** and **78** exhibited RIs and MS data close to those of  
136 khusian-2-ol and acorone respectively. However,  $^{13}\text{C}$  NMR analysis of the crude EOs did not  
137 permit to identify this two oxygenated sesquiterpenes. Thus, AP EO was submitted to CC  
138 yielding 16.2% of **72** in subfraction F<sub>AP4</sub> and 54.8% of **78** in F<sub>AP5-3</sub>. Compounds **72** and **78**  
139 were then isolated from F<sub>AP4</sub> and F<sub>AP5-3</sub> using pc-GC with respectively 95.8% and 82.4%  
140 purity (see Experimental).

141

#### 142 7-*epi*-Khusian-2-ol (**72**)

143 The combination of MS spectra and NMR data led to the molecular formula C<sub>15</sub>H<sub>26</sub>O.  
144 The  $^{13}\text{C}$  NMR spectrum displayed 15 signals whose multiplicities were deduced by DEPT  
145 135 and 90 as 3 C, 3 CH, 5 CH<sub>2</sub> and 4 CH<sub>3</sub>. The only characteristic  $^{13}\text{C}$  NMR value was a  
146 methine at 70.5 ppm. Taken altogether, MS and NMR data indicated that **72** is a tricyclic  
147 sesquiterpene alcohol. Here again, **72** exhibited  $^{13}\text{C}$  NMR values very similar to those of  
148 khusian-2-ol except for C-1, C-5 and C-13 ( $\Delta(\delta) > 3$  ppm) (Weyerstahl et al., 2000). These  
149 differences could be explained by a different relative configuration at C-7. Indeed, in the case  
150 of khusian-2-ol, the steric  $\gamma$  effect is observed between C-1 and C-13, whereas it appears  
151 between C-5 and C-13 for compound **72**. The C-7 stereochemistry of **72** was confirmed by the

152 NOESY correlation between H-5 and H-13 and the lack of the one between H-1 et H-13.  
153 Finally, **72** was identified as *7-epi-khusian-2-ol*. Total assignment of its <sup>1</sup>H and <sup>13</sup>C NMR  
154 spectra was achieved by means of 2D-NMR (Fig. 2, 3 and S2, Table 2).

155

#### 156 **4,8-di-*epi*-Acorone (78)**

157 MS and NMR data (DEPT 135 and 90) for **78** indicated a C<sub>15</sub>H<sub>24</sub>O<sub>2</sub> molecular formula  
158 and multiplicities of <sup>13</sup>C NMR signals as 3 C, 4 CH, 4 CH<sub>2</sub>, and 4 CH<sub>3</sub>. The two signals at  
159 209.7 ppm and 215.5 ppm indicated the presence of two ketone groups. The set of <sup>13</sup>C NMR  
160 values of **78** were similar to those of acorone, 1,8-di-*epi*-acorone (also named *epi*-acorone),  
161 and 1-*epi*-acorone (commonly named cryptoacorone) except for some carbons (Garneau et al.,  
162 2008; Nawamaki and Kuroyanagi, 1996). Thus, we focused our attention toward an epimer of  
163 acorone which <sup>1</sup>H and <sup>13</sup>C assignments were established through the accurate examination of  
164 HSQC, HMBC and COSY spectra. Concerning the relative configuration, the NOESY cross  
165 peak between hydrogens 3b and 6b indicated the stereochemistry of C-5. The NOESY  
166 correlation between H-3a and H-15 and the one between H-6a and H-13 showed that the two  
167 substituents of the cyclopentane were both oriented in a syn-periplanar manner. These  
168 previous observations allowed to deduce the relative configuration of C-5, C-1 and C-4. The  
169 NOESY cross peak of H-9b and H-10b with H-15 allowed to establish the inverted chair  
170 conformation of the cyclohexane. Finally, for minimal steric hindrance the methyl-14 was in  
171 the equatorial position. Consequently, compound **78** was identified as 4,8-di-*epi*-acorone (Fig.  
172 2, 3 and S2, Table 3).

173

#### 174 2.3.2. Structure Elucidation of 2-*epi*-Ziza-5-en-2-ol (**67**) and Antsorenone (**77**)

175 Compounds **67** and **77** eluted respectively at RIs 1582/2103 and 1749/2433 on both  
176 apolar and polar columns. These values did not match any constituents of our databases. In

177 order to identify these two molecules, the AP EO was subjected to several chromatography  
178 techniques (see Experimental).

179

### 180 **2-*epi*-Ziza-5-en-2-ol (67)**

181 Aerial parts EO containing 1.0% of **67** was chromatographed to yield F<sub>AP4</sub> (containing 3.7%  
182 of **67**), which was finally subjected to pc-GC to isolate **67** with 85.0% purity (GC). The  
183 difference of 500 points between the polar and apolar RI values, and the molecular ion peak  
184 in MS (*m/z* 220) suggested **67** was a sesquiterpene alcohol with a C<sub>15</sub>H<sub>24</sub>O molecular formula.  
185 By considering the degree of unsaturation and the carbon signals' multiplicities, compound **67**  
186 corresponded to an unsaturated tricyclic sesquiterpene alcohol. A DEPT experiment permitted  
187 to identify 5 C, 1 CH, 5 CH<sub>2</sub>, and 4 CH<sub>3</sub>. Moreover, we could deduce from the <sup>13</sup>C spectrum  
188 the presence of a tetrasubstituted double bond at 141.6 ppm/129.2 ppm and a tertiary alcohol  
189 at 80.3 ppm. Due to the similarities between our EOs with those of vetiver, and the presence  
190 of some prezizaane derivatives into *E. tristis* samples, we oriented our research towards a  
191 prezizaane/zizaane skeleton-based alcohol. Six out fifteen <sup>13</sup>C NMR signals (C-7, C-8, C-9,  
192 C-13, C-14 and C-15) were very similar to those of 2-*epi*-isozizaene and 12-norisoziza-5-ene  
193 (Filippi, 2014; Lin et al., 2006). Nevertheless, significant differences were observed for the  
194 rest of the signals mostly related to the α, β, and γ electronic effects induced by the alcohol  
195 function located on C-2. Thus, compound **67** was identified as 2-*epi*-ziza-5-en-2-ol (Fig. 2, 3  
196 and S2, Table 4).

197

### 198 **Antsorenone (77)**

199 Compound **77** was enriched up to 75.7% in F<sub>AP5-2</sub> by mean of repeated CC of *E.*  
200 *tristis* AP EO, and was finally purified up to 90.1% by pc-GC. The molecular formula of  
201 compound **77** was deduced according to MS and NMR data as C<sub>15</sub>H<sub>22</sub>O.

202 Additionally, its  $^{13}\text{C}$  NMR spectrum clearly showed the presence of a carbonyl function  
203 displaying interesting values ( $\delta_{\text{C}}$  209.2 (C-3)), and a double bond ( $\delta_{\text{C}}$  182.3 (C-1) and 130.9  
204 (C-2)), due to the mesomeric effect of the  $\alpha,\beta$ -unsaturated ketone, which are characteristic of  
205 an  $\alpha,\beta$ -unsaturated ketone in a cyclopentane ring (Pretsch et al., 2009).

206 This observation was confirmed by the long-range correlations between H-4a/4b and carbons  
207 C-1, C-2, C-3, and C-5. Moreover, MS and NMR data suggested that **77** was a tricyclic  
208 sesquiterpene ketone. Interestingly, C-12 resonance was shielded with a  $^{13}\text{C}$  NMR value of  
209 7.6 ppm (H-12 at 1.64 ppm) and was assigned to the methyl in  $\beta$  position to the ketone thanks  
210 to the HMBC correlations observed between H-12 and C-2/C-3.

211 The examination of other long-range  $^1\text{H}$ - $^{13}\text{C}$  correlations in the HMBC spectrum allowed to  
212 build two additional patterns:  $-\text{CH}(\text{CH}_3)-\text{C}(\text{CH}_3)_2-$  and  $-\text{CH}_2-\text{CH}-\text{CH}_2-\text{CH}_2-$ . The final  
213 tricyclic skeleton was first deduced from the connections of C-1/C-11 and C-5/C-10, and  
214 secondly of C-5/C-6 and C-7/C-8 giving a basic skeleton comparable to that previously  
215 observed for norsesquiterpene constituent of Indonesian vetiver oils, nigrítene and 6-*epi*-  
216 nigrítene (Champagnat et al., 2006; Filippi 2014). However, in contrast with that previous  
217 study, this ketone constitutes the first occurrence of a complete sesquiterpene framework (15  
218 carbons) for this skeleton. In agreement with its previous partial description by Chazan in  
219 1969, this ketone was named after the local name of the plant, Anstorenone (Chazan, 1969).

220 We thus propose the name Antsorane for this undescribed sesquiterpene family. The relative  
221 stereochemistry of this ketone could be easily deduced from the NOESY spectrum since all  
222 three aliphatic methyl groups displayed clear spatial correlations with specific hydrogens in  
223 the molecule. Thus, methyl-14 was correlated to H-9a, while methyl-15 was correlated to both  
224 H-6 and H-11a, suggesting thus the  $\alpha$ -orientation of methyl-13. Finally, the spatial correlation  
225 observed between H-10a and methyl-13 gave the ultimate clue to ensure the relative  
226 configuration of the entire structure (Fig. 2, 3 and S2, Table 5).

227

#### 228 2.4. Putative Biogenetic Relationships

229 While the biogenetic relationships between the acorane, helifolane, prezizaane, and  
230 zizaane skeletons are well known (Hong and Tantillo, 2009), the identification of compounds  
231 **50**, **67**, **69**, **71**, **72**, **77** and **78** helped to establish a complete formation pathway of these  
232 molecules in *E. tristis*. Most unexpectedly, the specific configuration observed at C<sub>f</sub>, C<sub>g</sub> and C<sub>j</sub>  
233 in the acorane skeleton (cyclopentane ring) appeared to remain untouched in all the  
234 succeeding sesquiterpenic skeletons. Hence, compounds **72** (helifolane), **50** and **69**  
235 (prezizaane), and **67** (isozizaane) were all displaying the same  $\alpha$ -orientation in position g as  
236 opposed, for instance, to what is naturally observed in vetiver oil constituents where methyl-g  
237 (or its functionalized version) is most of the time  $\beta$ -orientated. Moreover, the higher steric  
238 hindrance generated by the  $\alpha$ -orientation of methyl-g in the isozizaane skeleton, as seen for  
239 compound **67**, might have well triggered the Wagner-Meerwein transposition of the C<sub>d</sub>-C<sub>e</sub>  
240 bridge into position j, finally producing the newly identified antsorane skeleton.

241 Under acidic conditions, several attempts to transform **77** into zizaen-3-ones following  
242 a reverse Wagner-Merweein rearrangement failed. Several Brönsted and Lewis acids were  
243 tested but none of them succeeded in inducing the transposition. The high stability of **77** was  
244 extremely interesting, and it supported the hypothesis of the antsorane skeleton being the final  
245 step of the biogenetic pathway. Finally, it is worth mentioning that compound **77** was also  
246 detected in a solvent extract of *E. tristis* (made at room temperature with *n*-hexane). (Fig. 4).

247

### 248 3. Conclusion

249 Despite being a common plant used in folk medicine in Madagascar, *Elionurus tristis*, locally  
250 known as Antsoro, has rarely been investigated from a chemical standpoint. The aim of this

251 study was to establish the detailed chemical compositions of its essential oils extracted from  
252 both its aerial parts (AP) and its roots (R). The combined analysis of the two EOs by GC(RI),  
253 GC-MS and NMR allowed to respectively determine 93.8% and 97.2% of their entire  
254 chemical compositions in which 75 and 56 components were identified. This work also  
255 permitted to reveal the presence of several uncommon compounds such as  
256 cyclocopacamphene (**38**), 2-*epi*-prezizaene (**50**), 2-*epi*-prezizaan-7-ol (**69**), acorenone B (**71**),  
257 aristolone (**75**) and 1(10)-aristolen-2-one (**76**). Finally, four undescribed compounds could be  
258 entirely characterized: 2-*epi*-ziza-5-en-2-ol (**67**), 7-*epi*-khusian-2-ol (**72**), 4,8-di-*epi*-acorone  
259 (**78**), and antsorenone (**77**); this last one exhibits an undescribed natural sesquiterpene. Thanks  
260 to clear stereochemical relationships between the isolated constituents, our findings allowed  
261 us to decipher a probably active formation pathway of sesquiterpenes in *Elionurus tristis*,  
262 leading to the newly established antosrane skeleton.

## 263 **4. Experimental Part**

### 264 *4.1. Plant material, isolation of essential oils*

265 The essential oils were obtained by water distillation (for 4 hours) using a Clevenger-  
266 type apparatus from aerial parts (AP; 235.5 g) and roots (R; 481.5 g) of *Elionurus tristis*  
267 harvested at Lazaina Avaradrano (central region of Madagascar) on the 1<sup>st</sup> June 2015 (dry  
268 season). Both samples were obtained as light-yellow colored oils in 0.33% (771.3 mg) and  
269 0.21% (998.3 mg) yields, respectively. A voucher specimen of *E. tristis* has been deposited at  
270 the Malagasy Institute of Applied Research, (Antananarivo, Madagascar), under the accession  
271 number LHE015.

272

### 273 *4.2. Oils fractionation*

274 Aerial Parts oil: the essential oil obtained from the aerial parts of *Elionurus tristis*  
275 (771.3 mg) was separated into five fractions F<sub>AP1</sub>- F<sub>AP5</sub> by silica gel column chromatography  
276 (60–200 µm, 60 Å, 49.6 g) using an increasing gradient of pentane/Et<sub>2</sub>O 100/0 (F<sub>AP1</sub>=159.9  
277 mg), 95/5 (F<sub>AP2</sub>=86.4 mg), 90/10 (F<sub>AP3</sub>=157.8 mg), 80/20 (F<sub>AP4</sub>=151.1 mg) and 0/100  
278 (F<sub>AP5</sub>=212.6 mg).

279 Fraction F<sub>AP1</sub> contained **38** and **50** with the following contents: 4.7 and 13.0%.

280 Fraction F<sub>AP3</sub> contained **71** with 89.9% purity.

281 Fraction F<sub>AP4</sub> containing compounds **67** (3.7%), **69** (18.8%) and **72** (16.2%) and was directly  
282 used in pc-GC to purify the target compounds: **67** (85.0%), **69** (94.6%), **72** (95.8%). These  
283 three compounds were subject to NMR analysis for structure elucidation.

284 F<sub>AP5</sub> was further fractionated by CC with a finer silica gel (35–70 µm, 60 Å, 22.4 g) using  
285 pentane/acetone (93/7) to yield three subfractions: F<sub>AP5-1</sub>, 15.1 mg, F<sub>AP5-2</sub>, 120.7 mg, and  
286 F<sub>AP5-3</sub>, 71.9 mg. The last two subfractions respectively contained **77** (75.7%) and **78** (54.8%).  
287 Compounds **77** and **78** were finally isolated by pc-GC with respective purities of 90.1% and  
288 82.4% (Fig. S1).

289 Roots oil: the roots EO (998.3 mg) was subjected to the same procedure as above to  
290 provide five main fractions: F<sub>R1</sub>, 347.4 mg, F<sub>R2</sub>, 66.3 mg, F<sub>R3</sub>, 151.7 mg, F<sub>R4</sub>, 226.2 mg, and  
291 F<sub>R5</sub>, 129.7 mg. The last one was chromatographed a second time to provide F<sub>R5-1</sub> (45.1 mg),  
292 F<sub>R5-2</sub> (78.7 mg) and F<sub>R5-3</sub> (4.1 mg). The first two subfractions respectively contained **75**  
293 (82.5%) and **76** (56.9%).

294

295

### 296 4.3. Isomerization of Antsorenone (**77**)

297 Reactions were realized in a 50 mL round bottom flask equipped with a reflux  
298 condenser. Every assay was performed by taking approximately 15 mg ( $5.2 \cdot 10^{-2}$  mmol of **77**)

299 of F<sub>AP</sub>5-2 which were mixed with different acids in excess (see below) and heated to reflux  
300 for 6 hours under magnetic stirring as follows: (i) 1 mL of pure formic acid (26.0 mmol), (ii)  
301 1 mL of pure trifluoroacetic acid (12.9 mmol), (iii) 1 g of pure *p*TsOH.H<sub>2</sub>O (5.2 mmol)  
302 suspended in 5 mL of MeOH, (iv) a mixture of 1 g of pure *p*TsOH.H<sub>2</sub>O (5.2 mmol) and 1 mL  
303 of BF<sub>3</sub>.Et<sub>2</sub>O (7.8 mmol) was suspended in 7 mL of MeOH. After every assay, the acid was  
304 evaporated under reduced pressure. The residue was thus recovered by adding 3 mL of  
305 anhydrous Et<sub>2</sub>O, then washed by addition of NaHCO<sub>3</sub> until pH 7-8. The organic layer was  
306 separated and dried over MgSO<sub>4</sub>. The mixtures obtained for each of the four assays were  
307 directly analyzed by GC(RI) and GC-MS.

#### 308 4.4. *General Experimental Procedures*

309 Optical rotations were measured on a JASCO P-2000 polarimeter. UV and ECD  
310 spectra were recorded on a JASCO J-1500 spectrometer. IR spectra was recorded on a  
311 ThermoFisher SCIENTIFIC Nicolet iS5 FTIR spectrometer.

#### 312 4.5. *Gas Chromatography*

313 GC analyses were performed on a Clarus 500 PerkinElmer (PerkinElmer,  
314 Courtaboeuf, France) system equipped with a FID and two fused-silica capillary columns DB-  
315 1 (50m × 0.22 mm, film thickness 0.25 μm) and DB-WAX (50m × 0.22 mm, film thickness  
316 0.25 μm). The oven temperature was ramped from 60 °C to 220 °C at 2 °C/min and then held  
317 isothermal at 220 °C for 20 min; SSL inlet temperature: 250 °C; FID temperature: 250 °C;  
318 carrier gas: helium (0.8 mL/min); split: 1/60; injected volume: 0.5 μL. Retention indices (RI)  
319 were determined against a series of n-alkanes with linear interpolation (Target Compounds  
320 software from PerkinElmer). Quantification of the constituents was performed according to  
321 the method described by Tissot et al. (2012), (i) methyl octanoate (MO) was used as an  
322 internal reference; (ii) predicted relative response factors (PRRF) were calculated for each

323 compound; and (iii) the actual amount of each constituent was deduced and finally expressed  
324 in g/100 g.

325

#### 326 4.6. *Gas Chromatography-Mass Spectrometry in Electron Impact Mode*

327 Essential oils samples were analyzed with a Perkin-Elmer TurboMass detector  
328 (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL, equipped with two fused-  
329 silica capillary columns, DB-1 (50 m × 0.22 mm, film thickness 0.25 μm) and DB-WAX (50  
330 m × 0.22 mm, film thickness 0.25 μm). Carrier gas, helium at 0.8 mL/min; split: 1:70;  
331 injection volume: 0.5 μL; injector temperature: 250°C; oven temperature programmed from  
332 60°C to 220°C at 2°C/min and then held isothermal at 220 °C for 20 min. Ion source  
333 temperature: 250°C; energy ionisation: 70 eV; electron ionisation mass spectra were acquired  
334 over a 35-350 Da mass range.

335

#### 336 4.7. *Gas Chromatography-High Resolution Mass Spectrometry*

337 High-resolution EI-mass spectra were recorded using an Agilent 7200 GC-QTOF  
338 system (Agilent, Santa Clara, CA, USA), equipped with an Agilent J&W, DB-1 capillary  
339 column (30 m x 0.25 mm; 0.25 μm film thick). The mass spectrometer was operated at 70 eV  
340 with an acquisition rate of 2 GHz over a 35-450 *m/z* range, affording a resolution of ~8000.  
341 Injection volume 1 μL; split ratio 1/20; inlet temperature 250 °C, detector temperature 230  
342 °C; column flow (He) 1.2 mL/min; temperature program for oven 60 °C (5 min isotherm) to  
343 230 °C at 5 °C/min (5 min final isotherm).

344

#### 345 4.8. *Preparative capillary-Gas Chromatography*

346 Isolation of compounds **67**, **69**, **72**, **77** and **78** was performed using an Agilent 6890  
347 Plus gas chromatograph coupled to a Gerstel preparative fraction collector (PFC) operated  
348 under Chemstation Rev A.10.02/Gerstel Maestro 1.3.8.14. **67**, **69** and **72** were isolated on  
349 Phenomenex ZB-5 megabore capillary column (30 m × 0.53 mm; 3.0 μm film thick.). The  
350 oven temperature was ramped from 120 to 250 °C at 8 °C/min. Compounds **77** and **78** were  
351 isolated using a Supelcowax megabore capillary column (30 m × 0.53 mm; 0.5 μm film thick)  
352 with the following oven temperature programming: 140 to 250 °C at 10 °C/min. A Graphpack  
353 effluent splitter was connected to the column outlet, and was additionally mounted with 0.1  
354 mm and 0.32 mm deactivated fused-silica capillary restrictors (1 m each) to provide an  
355 FID/PFC ratio of ~1/9. The transfer line and the PFC were maintained at 230 °C. The injected  
356 volume was 1 μL in splitless mode. The system was operated in constant pressure mode at 35  
357 kPa. Compounds were trapped at room temperature in Gerstel U-type glass tubes by  
358 programming cutting times into the operating software allowing accurate automated  
359 operation. The isolation of any unknown compound in amounts sufficient for NMR analysis  
360 required 150–400 GC runs, and to avoid any contaminations, each product was collected  
361 directly in a NMR tube.

362

#### 363 4.9. Nuclear Magnetic Resonance

364 NMR spectra for compounds: (i) **67**, **69**, **72**, **78**, were recorded in C<sub>6</sub>D<sub>6</sub>; (ii) **77** was  
365 recorded in CDCl<sub>3</sub>; at 298 K on a Bruker Avance DRX 500 spectrometer operating at 500.13  
366 MHz for <sup>1</sup>H and 125.75 MHz for <sup>13</sup>C. In order to increase sensitivity, <sup>13</sup>C NMR spectra such  
367 as broadband-<sup>13</sup>C, DEPT 135 and DEPT 90 were run with a direct probe head (5 mm PADUL  
368 <sup>13</sup>C-<sup>1</sup>H Z-GRD). 1D- and 2D-NMR spectra such as <sup>1</sup>H, COSY, NOESY, HSQC, HMBC were  
369 run with an inverse probe head (5 mm PHTXI <sup>1</sup>H-<sup>13</sup>C/<sup>15</sup>N ZGRD). Spectrum calibration was

370 performed by using the C<sub>6</sub>D<sub>6</sub> signal (7.16 ppm for <sup>1</sup>H NMR, 128.06 ppm for <sup>13</sup>C NMR) or  
371 CDCl<sub>3</sub> signal (7.26 ppm for <sup>1</sup>H NMR, 77.16 ppm for <sup>13</sup>C NMR) as internal reference.  
372 Chemical shifts ( $\delta$ ) are expressed in parts per million (ppm) and coupling constants ( $J$ ) in  
373 hertz. All NMR experiments were carried out using pulse sequences supplied by the  
374 spectrometer manufacturer (Bruker Topspin<sup>TM</sup>) and processed via Mestrelab MestrelabNOVA  
375 software (Version 6.0.2-5475).

376 Other spectra (including **38**, **50**, **71**, **75**, **76**) were recorded on a Bruker AVANCE 400  
377 (100.623 MHz for <sup>13</sup>C) equipped with a 5-mm probe (DUL <sup>13</sup>C-<sup>1</sup>H Z-GRD Z8246/0066), in  
378 CDCl<sub>3</sub>. The <sup>1</sup>H NMR spectra were recorded with the following parameters, pulse width (PW),  
379 4.3 ms; acquisition time, 2.6 s for 32 K data table with a spectral width (SW) of 6000 Hz (15  
380 ppm). <sup>13</sup>C NMR spectra were recorded with the following parameters: pulse width (PW), 4  $\mu$ s  
381 (flip angle 45°); acquisition time, 2.7 s for 128 K data table with a spectral width of 24000 Hz  
382 (240 ppm); total repetition time, 2.8 s; CPD (composite pulse decoupling) mode decoupling;  
383 digital resolution, 0.183 Hz/point.

384

#### 385 *4.10. Identification and quantification of individual components*

386 Identification of individual components was based: a) on a comparison of their GC  
387 retention indice (RI) values on both polar and apolar stationary phases, with the literature  
388 (König et al., 2001). b) on computer search using digital libraries of mass spectral data and  
389 comparison with published data (Adams, 2007; McLafferty and Stauffer, 1994). c) on  
390 comparison of the signals in the <sup>13</sup>C NMR spectra of the mixtures with those of reference  
391 spectra compiled in the laboratory spectral library, with the help of a laboratory-made  
392 software (Ouattara et al., 2014; Tomi et al., 1995).

393

394 4.11. Spectral data

395

396 Cyclocopacamphene (**38**)

397  $C_{15}H_{14}$ ; RI (DB-1/DB-WAX) 1365/1479; EI-MS 70 eV,  $m/z$  (rel. int.): 204 (18,  $M^{+}$ ),  
398 189 (14,  $M^{+} - CH_3$ ), 175 (3), 162 (15), 161 (75,  $M^{+} - C_3H_7$ ), 147 (10), 135 (17), 134 (20),  
399 133 (41), 122 (18), 121 (28), 120 (43), 119 (81), 107 (45), 106 (25), 105 (100), 95 (21), 94  
400 (45), 93 (49), 92 (24), 91 (66), 81 (21), 79 (35), 77 (29), 69 (19), 67 (17), 65 (13), 55 (28), 53  
401 (15), 43 (18), 41 (42).  $^1H$  NMR ( $CDCl_3$ , 400 MHz) and  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz): see  
402 Table S1.

403

404 2-*epi*-Prezizaene (**50**)

405  $C_{15}H_{14}$ ; RI (DB-1/DB-WAX) 1447/1636; EI-MS 70 eV,  $m/z$  (rel. int.): 204 (4,  $M^{+}$ ),  
406 189 (22,  $M^{+} - CH_3$ ), 175 (8), 161 (24), 147 (21), 134 (35), 133 (93), 120 (19), 119 (51), 117  
407 (21), 108 (43), 107 (39), 105 (46), 95 (32), 93 (59), 91 (100), 81 (35), 79 (41), 77 (29), 69  
408 (21), 67 (30), 65 (14), 55 (31), 53 (18), 43 (10), 41 (32).  $^1H$  NMR ( $CDCl_3$ , 400 MHz) and  $^{13}C$   
409 NMR ( $CDCl_3$ , 100 MHz): see Table S1.

410

411 2-*epi*-Ziza-5-en-2-ol (**67**)

412 Colorless oily liquid;  $[\alpha]_D^{20} -28$  ( $c$  0.01,  $CH_3OH$ ); UV (MeOH)  $\lambda_{max}$  ( $\log \epsilon$ ) 192 (0.26)  
413 nm;  $C_{15}H_{24}O$ ; RI (DB-1/DB-WAX) 1582/2103; HREIMS:  $m/z$  220.1821 (calcd for  $C_{15}H_{24}O$ ,  
414 220.1827); EI-MS 70 eV,  $m/z$  (rel. int.): 220 (4,  $M^{+}$ ), 206 (5), 205 (36), 202 (8,  $M^{+} - H_2O$ ),  
415 188 (7), 187 (51), 177 (6), 160 (12), 159 (89), 149 (7), 147 (10), 145 (10), 133 (16), 131 (11),  
416 129 (6), 128 (6), 121 (6), 120 (10), 119 (100), 117 (10), 115 (10), 107 (8), 105 (20), 95 (6), 93  
417 (15), 91 (27), 81 (7), 79 (12), 77 (13), 69 (6), 67 (7), 65 (7), 55 (11), 53 (7), 43 (33).  $^1H$  NMR  
418 ( $C_6D_6$ , 500 MHz) and  $^{13}C$  NMR ( $C_6D_6$ , 125 MHz): see Table 4.

419

420 *2-epi-Prezizaan-7-ol (69)*

421 Colorless oily liquid;  $[\alpha]_D^{20}$  -14 (*c* 0.05, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 192 (0.58)  
422 nm; C<sub>15</sub>H<sub>26</sub>O; RI (DB-1/DB-WAX) 1640/2176; EI-MS 70 eV, *m/z* (rel. int.): 222 (5, M<sup>+</sup>),  
423 207 (3, M<sup>+</sup> – CH<sub>3</sub>), 204 (6, M<sup>+</sup> – H<sub>2</sub>O), 189 (19), 179 (28), 177 (8), 175 (8), 165 (4), 162 (5),  
424 161 (27), 151 (8), 148 (9), 147 (8), 138 (11), 137 (34), 135 (23), 134 (10), 133 (22), 123 (21),  
425 122 (11), 121 (25), 120 (9), 119 (53), 111 (9), 109 (48), 108 (14), 107 (32), 105 (24), 96 (12),  
426 95 (50), 94 (15), 93 (39), 91 (34), 84 (14), 83 (17), 82 (48), 81 (34), 79 (33), 77 (22), 71  
427 (100), 70 (14), 69 (34), 67 (51), 65 (10), 59 (12), 55 (36), 53 (15), 43 (91), 41 (35). <sup>1</sup>H NMR  
428 (C<sub>6</sub>D<sub>6</sub>, 500 MHz) and <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>, 125 MHz): see Table S2.

429

430 Acorenone B (71)

431 C<sub>15</sub>H<sub>24</sub>O; RI (DB-1/DB-WAX) 1665/2192; EI-MS 70 eV, *m/z* (rel. int.): 220 (10, M<sup>+</sup>),  
432 205 (3, M<sup>+</sup> – CH<sub>3</sub>), 191 (3), 177 (41, M<sup>+</sup> – C<sub>3</sub>H<sub>7</sub>), 163 (7), 150 (17), 149 (30), 136 (41), 135  
433 (82), 123 (26), 122 (20), 121 (61), 109 (100), 108 (37), 95 (26), 93 (53), 91 (35), 82 (67), 81  
434 (38), 79 (33), 77 (26), 69 (49), 67 (22), 55 (40), 54 (25), 53 (23), 43 (27), 41 (56). <sup>1</sup>H NMR  
435 (CDCl<sub>3</sub>, 400 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): see Table S3.

436

437 *7-epi-Khusian-2-ol (72)*

438 Colorless amorphous solid;  $[\alpha]_D^{20}$  +110 (*c* 0.08, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ )  
439 192 (0.57) nm; IR (ATR)  $\nu_{\max}$  3315, 2949, 2868, 1468, 1446, 1419, 1387, 1371, 1362, 1069,  
440 1035, 1021, 1009, 992 cm<sup>-1</sup>; C<sub>15</sub>H<sub>26</sub>O; RI (DB-1/DB-WAX) 1665/2313; HREIMS: *m/z*  
441 222.1979 (calcd for C<sub>15</sub>H<sub>26</sub>O, 222.1984); EI-MS 70 eV, *m/z* (rel. int.): 222 (12, M<sup>+</sup>), 207 (9,  
442 M<sup>+</sup> – CH<sub>3</sub>), 204 (3, M<sup>+</sup> – H<sub>2</sub>O), 191 (6), 189 (9), 179 (13), 178 (25), 177 (100), 166 (9), 165  
443 (7), 163 (17), 161 (28), 153 (9), 151 (24), 147 (13), 138 (21), 137 (12), 136 (11), 135 (29),

444 134 (8), 133 (19), 123 (25), 122 (55), 121 (50), 119 (50), 111 (22), 109 (43), 108 (16), 107  
445 (69), 105 (31), 96 (38), 95 (54), 93 (46), 91 (36), 84 (21), 83 (33), 82 (30), 81 (49), 79 (34),  
446 77 (23), 71 (24), 70 (12), 69 (44), 67 (36), 65 (11), 57 (23), 55 (67), 53 (20), 43 (52). <sup>1</sup>H NMR  
447 (C<sub>6</sub>D<sub>6</sub>, 500 MHz) and <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>, 125 MHz): see Table 2.

448

449 Aristolone (**75**)

450 C<sub>15</sub>H<sub>22</sub>O; RI (DB-1/DB-WAX) 1727/2427; EI-MS 70 eV, *m/z* (rel. int.): 218 (40, M<sup>+</sup>),  
451 204 (15), 203 (84, M<sup>+</sup> – CH<sub>3</sub>), 185 (6), 175 (42), 162 (20), 161 (41), 149 (15), 148 (17), 147  
452 (100), 145 (21), 135 (23), 134 (20), 133 (52), 131 (19), 122 (31), 121 (32), 119 (79), 117 (23),  
453 115 (21), 109 (44), 108 (31), 107 (36), 105 (60), 96 (22), 95 (23), 93 (32), 91 (77), 81 (25), 79  
454 (45), 77 (47), 69 (17), 67 (25), 65 (26), 55 (37), 53 (28), 43 (25). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  
455 and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): see Table S4.

456

457 1(10)-Aristolen-2-one (**76**)

458 C<sub>15</sub>H<sub>22</sub>O; RI (DB-1/DB-WAX) 1730/2395; EI-MS 70 eV, *m/z* (rel. int.): 218 (8, M<sup>+</sup>),  
459 203 (30, M<sup>+</sup> – CH<sub>3</sub>), 185 (37), 177 (10), 176 (77), 175 (66), 162 (16), 161 (97), 157 (8), 148  
460 (16), 147 (68), 145 (11), 143 (16), 135 (26), 134 (18), 133 (67), 131 (18), 121 (31), 120 (13),  
461 119 (60), 117 (20), 115 (20), 107 (32), 106 (17), 105 (100), 103 (12), 95 (10), 93 (29), 92  
462 (11), 91 (77), 81 (12), 79 (34), 78 (12), 77 (46), 69 (41), 67 (29), 65 (28), 55 (36), 53 (25), 51  
463 (13), 43 (25), 41 (70). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): see  
464 Table S1.

465

466 Antsorenone (**77**)

467 Yellow oily liquid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +79 (*c* 0.21, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 190-270  
468 (1.00) nm; C<sub>15</sub>H<sub>22</sub>O; RI (DB-1/DB-WAX) 1749/2433; HREIMS: *m/z* 218.1668 (calcd for

469 C<sub>15</sub>H<sub>22</sub>O, 218.1671); EI-MS 70 eV, *m/z* (rel. int.): 218 (32, M<sup>+</sup>), 203 (16, M<sup>+</sup> – CH<sub>3</sub>), 189  
470 (6), 176 (6), 175 (23), 162 (16), 161 (20), 149 (18), 148 (100), 147 (90), 134 (18), 133 (35),  
471 130 (12), 122 (14), 121 (12), 120 (50), 119 (43), 117 (14), 115 (12), 110 (12), 109 (34), 108  
472 (13), 106 (17), 105 (65), 97 (7), 93 (10), 92 (17), 91 (60), 79 (23), 78 (11), 77 (30), 71 (28),  
473 69 (12), 67 (14), 65 (17), 57 (8), 55 (43), 53 (20), 51 (8), 43 (36). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500  
474 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): see Table 5.

475

476 4,8-di-*epi*-Acorone (**78**)

477 Yellow oily liquid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -51 (*c* 0.04, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 192 (0.58)  
478 nm; C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>; RI (DB-1/DB-WAX) 1765/2553; HREIMS: *m/z* 236.1772 (calcd for C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>,  
479 236.1776); EI-MS 70 eV, *m/z* (rel. int.): 236 (8, M<sup>+</sup>), 221 (3, M<sup>+</sup> – CH<sub>3</sub>), 203 (8), 193 (13,  
480 M<sup>+</sup> – C<sub>3</sub>H<sub>7</sub>), 175 (4), 166 (25), 165 (12), 161 (4), 152 (14), 151 (100), 147 (14), 138 (74), 137  
481 (26), 136 (12), 133 (6), 124 (20), 123 (53), 121 (12), 111 (23), 110 (17), 109 (45), 107 (11),  
482 96 (12), 95 (35), 93 (13), 91 (12), 85 (8), 83 (21), 82 (14), 81 (43), 79 (22), 77 (14), 69 (68),  
483 67 (42), 57 (11), 55 (44), 53 (22), 43 (35), 42 (25). <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 500 MHz) and <sup>13</sup>C NMR  
484 (C<sub>6</sub>D<sub>6</sub>, 125 MHz): see Table 3.

485

## 486 Supplementary Materials

487

488 The following data are available online. Tables S1-S4 and Figures S1-S20:

489 Fractionation scheme of *E. tristis* essential oil (AP) and isolation of some constituents; EI-MS  
490 spectra for compounds **38**, **50**, **67**, **69**, **71**, **72**, **75**, **76**, **77**, **78**; UV and CD spectra for  
491 compounds **67**, **69**, **72**, **77** and **78**; FT-IR spectrum for compound **72**; <sup>13</sup>C-NMR data for  
492 compounds **38**, **50** and **76**; 1D- and 2D-NMR data for compounds **69**, **71** and **75**; 1D- and 2D-  
493 NMR spectra for compounds **67**, **69**, **71**, **72**, **75**, **77** and **78**.

494

495

496

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502

503

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582

**Table 1.** Chemical composition of essential oils of *Elionurus tristis*.

#	Compound name	RI lit <sup>a</sup>	RI DB-1	RI WAX	PRRF <sub>i,MO</sub>	Aerial Parts g/100g	Roots g/100g	Id. methods
1	Tricyclene	927	919	1013	0.765	1.0	3.8	RI, MS, <sup>13</sup> C NMR
2	$\alpha$ -Thujene	932	927	1013	0.765	tr	0.1	RI, MS
3	$\alpha$ -Pinene	936	929	1013	0.765	1.7	7.3	RI, MS, <sup>13</sup> C NMR
4	Camphene	950	942	1066	0.765	4.0	13.6	RI, MS, <sup>13</sup> C NMR
5	6-Methyl-hept-5-en-2-one	978	959	1337	0.917	0.1	0.1	RI, MS
6	Sabinene	973	963	1122	0.765	tr	0.2	RI, MS
7	$\beta$ -Pinene	978	969	1110	0.765	tr	0.2	RI, MS
8	Myrcene	987	979	1163	0.765	0.5	0.3	RI, MS, <sup>13</sup> C NMR
9	<i>p</i> -Cymene	1015	1010	1274	0.698	0.3	0.7	RI, MS, <sup>13</sup> C NMR
10	Limonene*	1025	1019	1203	0.765	0.5	1.6	RI, MS, <sup>13</sup> C NMR
11	1,8-Cineole*	1024	1019	1213	0.869	1.0	3.2	RI, MS, <sup>13</sup> C NMR
12	Camphenilone	-	1056	-	0.912	0.1	-	RI, MS
13	Nonanal	1076	1080	1392	0.870	tr	-	RI, MS
14	Linalool	1086	1082	1548	0.869	0.4	-	RI, MS
15	Perillene	1090	1084	1419	0.907	tr	-	RI, MS
16	Campholenal A	1105	1104	1481	0.887	tr	-	RI, MS
17	Camphor	1123	1119	1519	0.887	0.1	0.2	RI, MS
18	<i>trans</i> -Pinocarveol	1126	1122	1650	0.876	tr	0.1	RI, MS
19	<i>trans</i> -Verbenol	1136	1128	1680	0.876	0.1	tr	RI, MS
20	Camphene hydrate	1143	1130	1596	0.869	tr	0.1	RI, MS
21	<i>p</i> -Mentha-1,5-dien-8-ol	1127	1147	1728	0.887	0.2	-	RI, MS
22	Borneol	1150	1151	1701	0.869	0.3	0.4	RI, MS
23	<i>p</i> -Methylacetophenone	1156	1152	1779	0.839	tr	0.2	RI, MS
24	Cryptone	1160	1153	1661	0.912	0.2	0.2	RI, MS
25	<i>p</i> -Cymen-8-ol	1169	1158	1803	0.809	0.1	0.1	RI, MS
26	Terpinen-4-ol	1164	1160	1597	0.869	0.1	0.3	RI, MS
27	Myrtenal	1172	1167	1627	0.907	tr	tr	RI, MS
28	$\alpha$ -Terpineol	1176	1170	1697	0.869	0.3	0.5	RI, MS, <sup>13</sup> C NMR
29	Verbenone	1183	1179	1708	0.907	0.2	0.2	RI, MS
30	Decanal	1180	1182	1499	0.852	0.1	-	RI, MS
31	Octyl acetate	1188	1195	1473	0.974	0.1	-	RI, MS
32	<i>cis</i> -Carveol	1210	1213	1835	0.887	0.1	-	RI, MS
33	Carvone	1214	1214	1739	0.907	tr	0.1	RI, MS
34	Piperitone	1226	1224	1728	0.887	0.1	0.1	RI, MS
35	Decanol	1264	1253	1760	0.836	0.2	-	RI, MS
36	Bornyl acetate	1270	1267	1581	0.958	2.7	3.3	RI, MS, <sup>13</sup> C NMR
37	<i>trans</i> -Pinocarvyl acetate	1287	1289	1648	0.976	0.1	0.1	RI, MS
38	Cyclocopacamphene	1365 <sup>b</sup>	1365	1479	0.751	1.6	2.2	RI, MS, <sup>13</sup> C NMR
39	Cyclocosativene	1367 <sup>b</sup>	1367	1479	0.751	tr	0.1	RI, MS
40	$\alpha$ -Ylangene	1376	1373	1472	0.751	0.2	-	RI, MS
41	$\alpha$ -Funebrene	1385	1384	1500	0.751	0.2	-	RI, MS

42	7- <i>epi</i> -Sesquithujene	1387	1390	1527	0.751	tr	-	RI, MS
43	Sativene	1394	1392	1523	0.751	tr	-	RI, MS
44	$\alpha$ -Cedrene	1418	1415	1566	0.751	1.8	1.6	RI, MS, <sup>13</sup> C NMR
45	( <i>E</i> )- $\beta$ -Caryophyllene	1421	1418	1571	0.751	0.1	-	RI, MS
46	<i>trans</i> - $\alpha$ -Bergamotene*	1434	1430	1584	0.751	7.0	3.3	RI, MS, <sup>13</sup> C NMR
47	Calarene*	1437	1430	1591	0.751	2.1	12.7	RI, MS, <sup>13</sup> C NMR
48	Sesquisabinene A	1435	1433	1647	0.751	1.5	0.6	RI, MS, <sup>13</sup> C NMR
49	Prezizaene	1452	1442	1630	0.751	0.8	1.3	RI, MS, <sup>13</sup> C NMR
50	2- <i>epi</i> -Prezizaene	-	1447	1636	0.751	4.0	6.3	RI, MS, <sup>13</sup> C NMR
51	$\beta$ -Santalene	1460	1452	1646	0.751	0.7	0.8	RI, MS, <sup>13</sup> C NMR
52	Dauca-5,8-diene	-	1465	1654	0.751	-	0.2	RI, MS
53	$\alpha$ -Curcumene	1473	1467	1773	0.707	1.1	0.6	RI, MS, <sup>13</sup> C NMR
54	$\alpha$ -Amorphene	1477	1471	1686	0.751	0.5	0.8	RI, MS, <sup>13</sup> C NMR
55	<i>trans</i> - $\beta$ -Bergamotene	1480	1476	1682	0.751	1.6	0.7	RI, MS, <sup>13</sup> C NMR
56	$\beta$ -Bisabolene	1503	1497	1720	0.751	0.1	tr	RI, MS
57	$\beta$ -Curcumene	1503	1499	1734	0.751	0.1	-	RI, MS
58	$\alpha$ -Alaskene	1512	1505	-	0.751	-	0.2	RI, MS
59	( <i>Z</i> )- $\gamma$ -Bisabolene	1505	1505	1732	0.751	0.5	-	RI, MS, <sup>13</sup> C NMR
60	<i>cis</i> -Calamenene	1517	1506	1826	0.707	0.2	0.2	RI, MS
61	7- <i>epi</i> - $\alpha$ -Selinene	1519	1512	1760	0.751	-	0.1	RI, MS
62	$\delta$ -Cadinene	1520	1512	1748	0.751	0.6	-	RI, MS, <sup>13</sup> C NMR
63	Sesquiphellandrene B	1516	1514	1773	0.751	0.7	0.5	RI, MS, <sup>13</sup> C NMR
64	$\alpha$ -Calacorene	1527	1525	-	0.715	0.1	0.1	RI, MS
65	( <i>E</i> )-Nerolidol	1553	1545	2037	0.819	1.1	-	RI, MS, <sup>13</sup> C NMR
66	Caryophyllene oxide	1578	1566	1980	0.830	4.0	0.7	RI, MS, <sup>13</sup> C NMR
67	2- <i>epi</i> -Ziza-5-en-2-ol	-	1582	2103	0.830	1.0	1.3	RI, MS, 2D-NMR
68	1,10-di- <i>epi</i> -Cubenol	1615	1614	2054	0.819	0.2	0.3	RI, MS
69	2- <i>epi</i> -Prezizaan-7-ol	-	1640	2176	0.819	5.1	1.8	RI, MS, 2D-NMR
70	$\beta$ -Bisabolol	1659	1652	2142	0.819	0.6	-	RI, MS
71	Acorenone B*	-	1665	2192	0.830	17.6	7.2	RI, MS, 2D-NMR
72	7- <i>epi</i> -Khusian-2-ol*	-	1665	2313	0.819	3.5	1.5	RI, MS, 2D-NMR
73	Acora-3,7(11)-dien-8-one	1709	1695	-	0.841	0.2	-	RI, MS
74	Bisabolone	1750 <sup>c</sup>	1715	-	0.830	0.2	-	RI, MS
75	Aristolone	-	1727	2427	0.841	0.6	3.1	RI, MS, 2D-NMR
76	1(10)-Aristolen-2-one	-	1730	2395	0.841	1.3	6.4	RI, MS, <sup>13</sup> C NMR
77	Antsorenone	-	1749	2433	0.841	15.4	5.6	RI, MS, 2D-NMR
78	4,8-di- <i>epi</i> -Acorone	-	1765	2553	0.913	3.1	0.2	RI, MS, 2D-NMR
<b>Total</b>						<b>93.8</b>	<b>97.2</b>	

Order of elution and percentages are given on apolar column; except for those with an asterisk (\*), percentages on polar column. RI<sub>a</sub>, RI<sub>p</sub>: retention indices measured on apolar (DB-1) and polar (DB-WAX) columns, respectively. <sup>a</sup>RI lit: retention indices according to Tepenoids Library Website (König et al., 2001). <sup>b</sup>RI lit: retention index according to Garcia et al. (2017). <sup>c</sup>RI lit: retention index according to Mevy et al. (2002). The relative proportions of each constituent were expressed in g/100 g. tr: traces (<0.05%); 2D-NMR = 2D-NMR spectra are provided in Supporting Information.

**Table 2.** NMR data of 7-*epi*-Khusian-2-ol (**72**).

C	<sup>13</sup> C δ (ppm)	<sup>1</sup> H	<sup>1</sup> H δ (ppm) by HSQC	COSY <sup>1</sup> H- <sup>1</sup> H	HMBC H → C	NOESY <sup>a</sup>
1	45.1	1a 1b	1.47-1.52 (m) 1.36 (dd, <i>J</i> = 12.9 5.4 Hz)	1b, 2, 5 1a, 2	2, 3, 5, 6 2, 5, 6, 10	2 -
2	70.5	2	3.78 (ddd, <i>J</i> = 9.6 5.4 2.2 Hz)	1a, 1b, 4b	4	1a, <u>12</u> , <u>14</u>
3	39.9	-	-	-	-	-
4	23.5	4a 4b	1.64-1.70 (m) 1.15-1.21 (m)	4b, 5 2, 4a, 5	2, 3, 5, 11 2, 3, 5	4b, 5, <u>12</u> 4a, <u>12</u>
5	20.4	5	1.00-1.08 (m)	1a, 4a, 4b	1, 4, 6, 7, 10	4a, <u>13</u>
6	43.2	-	-	-	-	-
7	42.7	7	1.31-1.36 (m)	8a, 8b, 13	5, 6, 8, 13	8a, 13
8	32.2	8a 8b	1.70-1.78 (m) 1.13-1.19 (m)	7, 8b, 9 7, 8a, 9	7, 9, 10 7, 9, 13	7, 8b, 9 8a
9	22.6	9	1.39-1.47 (m)	8a, 8b, 10	8, 10, 11	8a, <u>15</u>
10	54.3	10	1.12-1.19 (m)	9	1, 5, 6, 9, 11, 14, 15	-
11	34.9	-	-	-	-	-
12	16.5	12	0.72 (s)	-	2, 3, 4, 11	<u>2</u> , <u>4a</u> , <u>4b</u>
13	14.6	13	0.77 (d, <i>J</i> = 6.9 Hz)	7	6, 7, 8	<u>5</u> , 7
14	26.8	14	0.78 (s)	-	3, 10, 11, 15	<u>2</u>
15	21.7	15	0.80 (s)	-	3, 10, 11, 14	<u>9</u>

<sup>a</sup>Pure NOE correlations appear underlined. Hydrogens H1a/H1b, H4a/H4b and H8a/H8b could not be differentiated.

**Table 3.** NMR data of 4,8-di-*epi*-Acorone (**78**).

C	<sup>13</sup> C δ (ppm)	<sup>1</sup> H	<sup>1</sup> H δ (ppm) by HSQC	COSY <sup>1</sup> H- <sup>1</sup> H	HMBC H → C	NOESY <sup>a</sup>
1	59.6	1	1.64-1.67 (m)	-	2, 5, 6, 10, 11, 12	<u>13</u>
2	215.5	-	-	-	-	-
3	45.0	3a ( <i>anti</i> ) 3b ( <i>syn</i> )	1.57 (d, <i>J</i> = 18.6 Hz) 2.17 (dd, <i>J</i> = 18.6 8.3 Hz)	3b 3a, 4	2, 15 2, 4, 15	3b, <u>15</u> 3a, 4, <u>6a</u> , <u>6b</u>
4	32.2	4	1.64-1.69 (m)	3b, 15	2, 3, 5, 6, 10, 15	3b, <u>6b</u> , 15
5	50.5	-	-	-	-	-
6	47.9	6a 6b	1.89 (dd, <i>J</i> = 12.8 1.0 Hz) 2.05 (dd, <i>J</i> = 12.8 2.8 Hz)	6b 6a, 10b	1, 4, 5, 7, 10 4, 5, 7, 8, 10	<u>3b</u> , 6b, <u>10a</u> , <u>11</u> , <u>12</u> , <u>13</u> <u>3b</u> , <u>4</u> , 6a, <u>12</u>
7	209.7	-	-	-	-	-
8	44.3	8	1.80 (m, <i>J</i> = 6.4 Hz)	9a, 14	7, 9, 14	9a, 14
9	31.0	9a 9b	1.43 (m) 1.01-1.06 (m)	8, 9b 9a, 10a	7, 8, 10 8, 10, 14	8, 9b 9a, 10a, <u>15</u>
10	31.5	10a 10b	1.27-1.32 (m) 1.13-1.19 (m)	9b, 10b 6b, 10a	4, 5, 6, 8, 9, 11 6, 8, 9	<u>6a</u> , 9b, 10b 10a, <u>15</u>
11	25.3	11	1.57 (m)	12, 13	1, 2, 5, 12, 13	<u>6a</u> , 12, 13
12	19.7	12	0.95 (d, <i>J</i> = 6.8 Hz)	11	1, 11, 13	<u>6a</u> , <u>6b</u> , 11
13	24.9	13	1.16 (d, <i>J</i> = 7.0 Hz)	11	1, 11, 12	<u>1</u> , <u>6a</u> , 11
14	14.7	14	1.01 (d, <i>J</i> = 6.5 Hz)	8	7, 8, 9, 10	8
15	15.1	15	0.36 (d, <i>J</i> = 7.2 Hz)	4	3, 4, 5	<u>3a</u> , 4, <u>9b</u> , <u>10b</u>

<sup>a</sup>Pure NOE correlations appear underlined.

**Table 4.** NMR data of 2-*epi*-Ziza-5-en-2-ol (**67**).

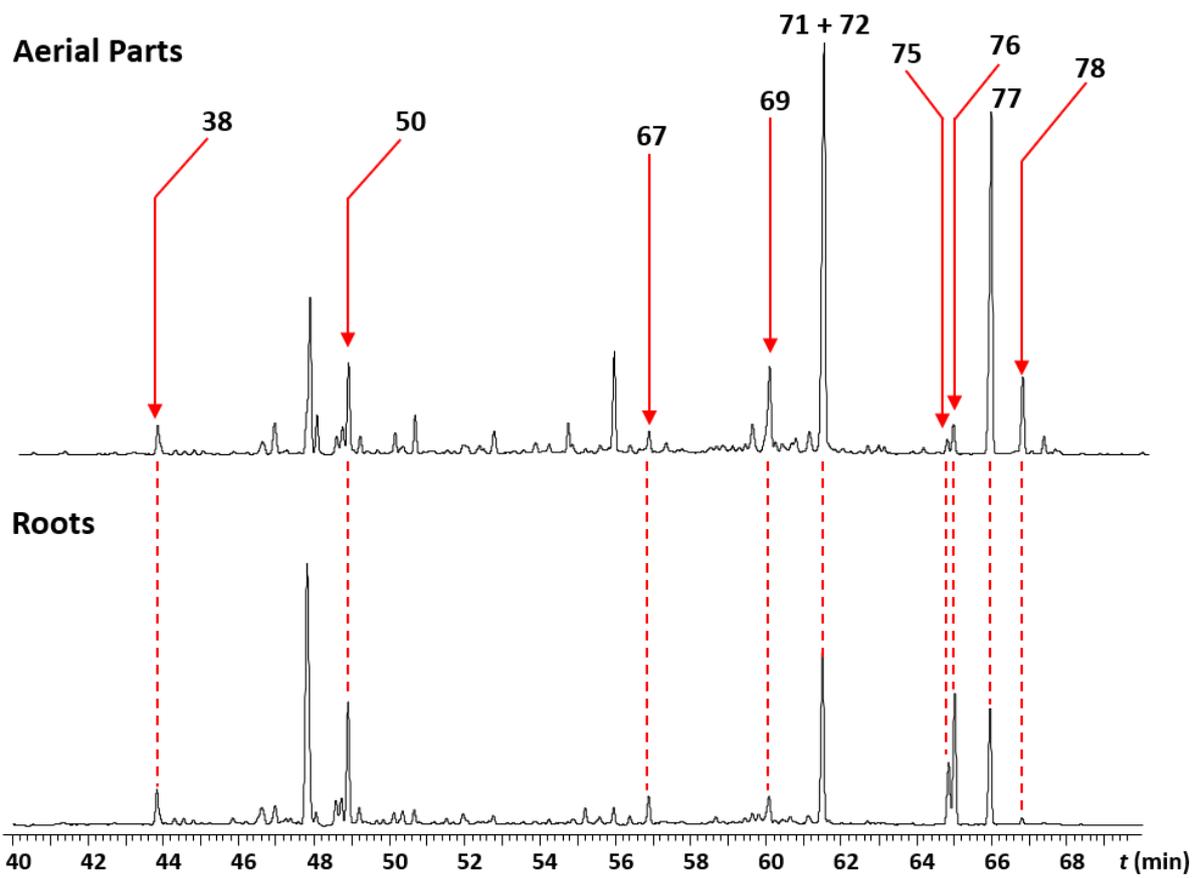
C	<sup>13</sup> C δ (ppm)	<sup>1</sup> H	<sup>1</sup> H δ (ppm) by HSQC	COSY <sup>1</sup> H- <sup>1</sup> H	HMBC H → C	NOESY <sup>a</sup>
1	58.2	-	-	-	-	-
2	80.3	-	-	-	-	-
3	38.9	3a ( <i>anti</i> ) 3b ( <i>syn</i> )	1.55 (m) 1.67 (m)	3b, 4a, 4b 3a, 4a	4 1, 2, 4, 5	4a, 4b, <u>10a</u> , <u>12</u> 4a
4	25.7	4a 4b	2.28-2.40 (m) 2.12 (dd, <i>J</i> = 17.0 9.0 Hz)	3a, 3b, 4b, 13 3a, 4a, 13	5 2, 5, 6	3a, 3b, 4b, 13 3a, 4a
5	141.6	-	-	-	-	-
6	129.2	-	-	-	-	-
7	40.8	-	-	-	-	-
8	47.8	8	1.81-1.86 (m)	9b, 10b, 11b	1, 6, 7, 10, 11	10b, 11b, <u>14</u> , <u>15</u>
9	25.5	9a ( <i>exo</i> ) 9b ( <i>endo</i> )	1.80-1.85 (m) 1.56-1.63 (m)	9b, 10b, 11a, 11b 8, 9a, 10a, 10b, 11a, 11b	1, 7, 8, 10, 11, 14 -	9b, 10b 9a, 10b, <u>15</u>
10	32.4	10a ( <i>exo</i> ) 10b ( <i>endo</i> )	1.77-1.83 (m) 1.27-1.33 (m)	9b, 10b, 11a, 11b 8, 9a, 9b, 10a, 11b	8, 9, 11 1, 5, 7, 8, 12	<u>3a</u> , 10b, <u>12</u> 8, 9a, 9b, 10a
11	34.7	11a ( <i>exo</i> ) 11b ( <i>endo</i> )	1.32-1.38 (m) 1.15-1.22 (m)	9a, 9b, 10a, 11b 8, 9a, 9b, 10a, 10b, 11a	1, 5, 7, 9 14	11b, <u>12</u> 8, 11a
12	22.3	12	1.13 (s)	-	1, 2, 3	<u>3a</u> , <u>10a</u> , <u>11a</u>
13	13.3	13	1.46 (s)	4a, 4b	5, 6, 7	4a, <u>14</u> , <u>15</u>
14	28.6	14	1.05 (s)	-	6, 7, 8, 15	<u>8</u> , <u>13</u>
15	25.3	15	0.99 (s)	-	6, 7, 8, 14	<u>8</u> , <u>9b</u> , <u>13</u>

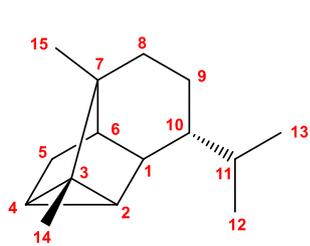
<sup>a</sup>Pure NOE correlations appear underlined. Hydrogens H4a/H4b could not be differentiated

**Table 5.** NMR data of Antsorenone (**77**).

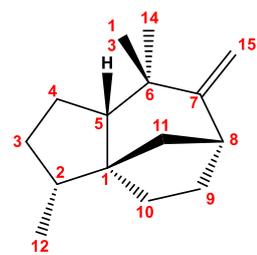
C	<sup>13</sup> C δ (ppm)	<sup>1</sup> H	<sup>1</sup> H δ (ppm) by HSQC	COSY <sup>1</sup> H- <sup>1</sup> H	HMBC H → C	NOESY <sup>a</sup>
1	182.3	-	-	-	-	-
2	130.9	-	-	-	-	-
3	209.2	-	-	-	-	-
4	45.5	4a	2.16 (dd, <i>J</i> = 18.5 1.2 Hz)	4b, 11a	1, 3, 5, 10	4b, <u>6</u> , <u>13</u>
		4b	1.97 (dd, <i>J</i> = 18.5 1.3 Hz)	4a	1, 2, 3, 5, 10	4a
5	44.4	-	-	-	-	-
6	44.7	6	1.26 (qd, <i>J</i> = 7.6 1.8 Hz)	13	1, 5, 7, 10, 13, 15	<u>4a</u> , 13, <u>15</u>
7	34.5	-	-	-	-	-
8	38.6	8	1.51 (quintuplet, <i>J</i> = 3.0 Hz)	9a, 9b, 11a, 11b	1, 6, 7, 9, 10, 11, 15	9a, 11a, 11b, <u>14</u> , <u>15</u>
9	23.6	9a	1.89-1.97 (m)	8, 9b, 10a, 10b, 11a	5, 8, 11	8, 9b, <u>14</u>
		9b	1.33-1.41 (m)	8, 9a, 10a, 10b	5, 7, 8, 10, 11	9a, 10b, <u>11b</u>
10	24.5	10a	1.95-2.04 (m)	9a, 9b, 10b	1, 5, 6, 9	10b, <u>13</u>
		10b	1.07-1.15 (m)	9a, 9b, 10a	1, 5, 6, 8, 13, 15	9b, 10a
11	29.9	11a	2.83 (dm, <i>J</i> = 19.5 Hz)	4a, 8, 9a, 11b, 12	1	8, 11b, 12, <u>15</u>
		11b	2.35 (dm, <i>J</i> = 19.5 Hz)	8, 11a, 12	1, 7	8, <u>9b</u> , 11a, 12
12	7.6	12	1.64 (t, <i>J</i> = 1.4 Hz)	11a, 11b	1, 2, 3, 5, 10, 11	11a, 11b
13	12.0	13	0.95 (d, <i>J</i> = 7.3 Hz)	6	5, 6, 7, 8, 14	<u>4a</u> , 6, <u>10a</u>
14	24.8	14	0.99 (s)	-	6, 7, 8, 13, 15	<u>8</u> , <u>9a</u>
15	30.9	15	0.95 (s)	-	6, 7, 8, 14	<u>6</u> , <u>8</u> , <u>11a</u>

<sup>a</sup>Pure NOE correlations appear underlined.

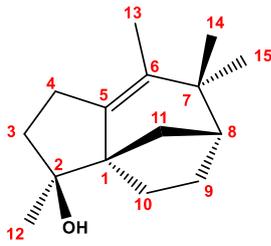




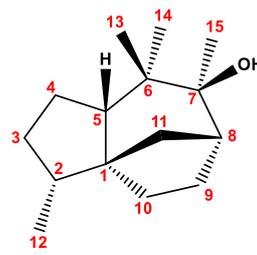
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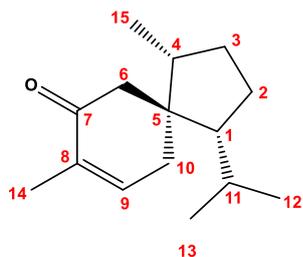
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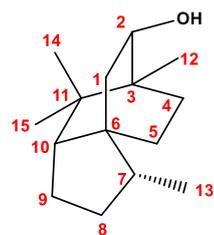
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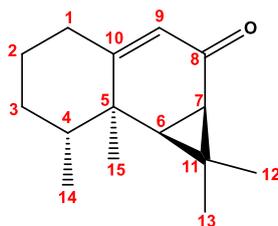
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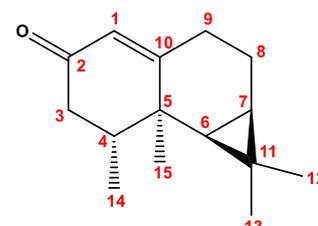
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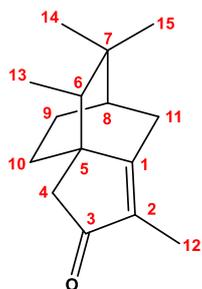
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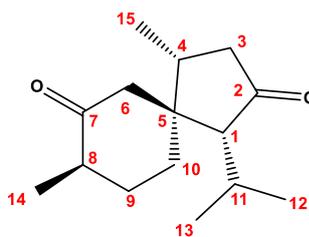
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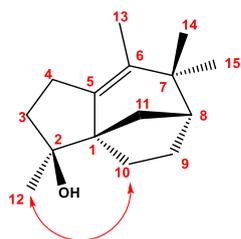
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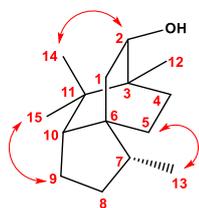
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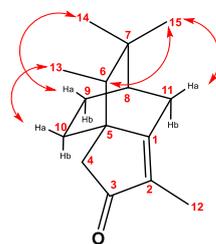
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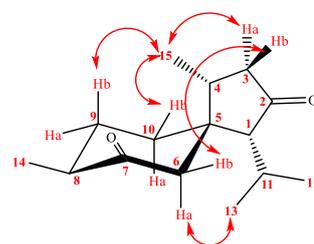
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**67**



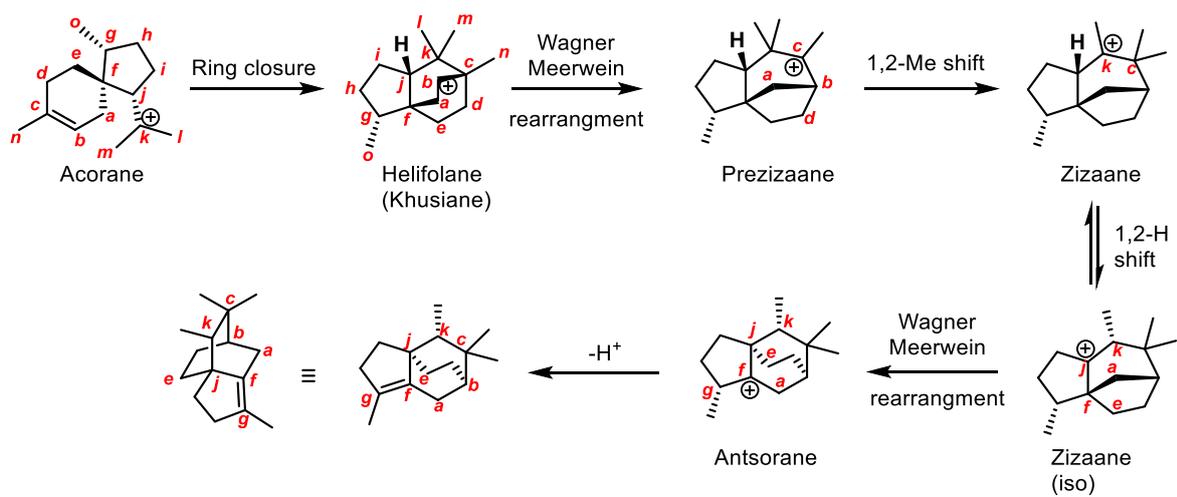
7-*epi*-Khusian-2-ol  
**72**



Antsorenone  
**77**



4,8-di-*epi*-Acorone  
**78**



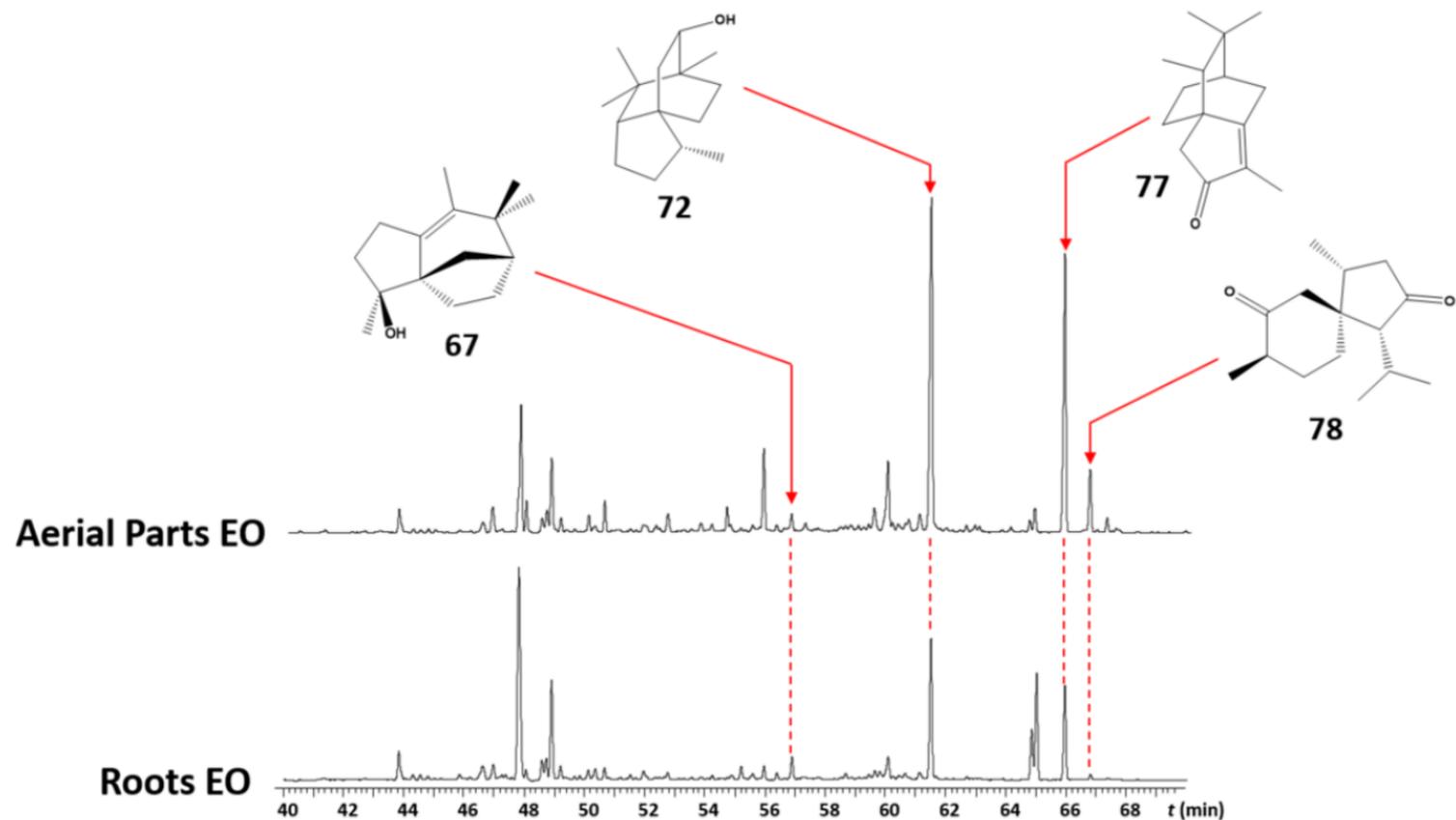
**Fig. 1.** Gas chromatograms on apolar column of the Aerial Parts and the Roots essential oils depicting compounds **38**, **50**, **67**, **69**, **71**, **72**, **75**, **76**, **77**, **78**

**Fig. 2.** Structures of compounds characterized by NMR. Compounds **67**, **69**, **72**, **77**, and **78** were isolated by pc-GC.

**Fig. 3.** Key-NOEs for isolated compounds **67**, **72**, **77**, and **78**.

**Fig. 4.** Putative formation pathway of the antsorane skeleton.

# *Elionurus tristis* Hack. from Madagascar



- The composition of the essential oils was analyzed by GC, GC-MS, pc-GC and NMR spectroscopy.
- GC of the essential oils depicting compounds **67**, **72**, **77** and **78**.