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## **Chemical constituents of non-polar fractions obtained from *Cnidoscolus quercifolius* Pohl (Euphorbiaceae), a Brazilian medicinal plant native to Caatinga biome**

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## **Constituintes químicos de frações não-polares obtidas de *Cnidoscolus quercifolius* Polh (Euphorbiaceae), uma planta medicinal brasileira nativa da Caatinga**

**Abstract:** *Cnidoscolus quercifolius* Pohl is a Brazilian medicinal plant from Caatinga biome and possesses several pharmacological properties. In this paper, we describe the identification of compounds from non-polar fractions of the leaves (Hex-Le) and stem-barks (Hex-Sb) from *C. quercifolius*. Chemical analysis were performed by GC-MS approach. Chemical constituents were identified based on spectral data obtained and by comparison with literature data. Fragmentation profile of the main compounds was proposed and presented in this paper. Hydrocarbons (46.84%), triterpenes (32.60%) and carotenoid derivatives (0.83%) were considered the major constituent of Hex-Le. In contrast, hydrocarbons (41.89%), diterpenes (12.83%) and triterpenes (7.0%) were found in Hex-Sb. Four diterpenes (dehydroabietane, sandaracopimaradiene, kaur-16-ene and 13-methyl-17-norkaur-15-ene) and one triterpene (diploptene) are reported for the first time in *Cnidoscolus*.

**Keywords:** medicinal plants; Caatinga; terpenoids; *Cnidoscolus*.

**Resumo:** *Cnidoscolus quercifolius* Pohl é uma planta medicinal do Brasil, nativa do bioma Caatinga, que apresenta diversas propriedades farmacológicas. Nesse estudo, nós descrevemos a identificação de compostos de frações não-polares obtidas das folhas (Hex-Le) e cascas do caule (Hex-Sb) da espécie. As análises químicas foram realizadas por CG-EM. Os constituintes químicos foram identificados com base nos dados espectrais obtidos e por comparação com os dados da literatura. O perfil de fragmentação dos principais compostos identificados também foi proposto e é igualmente apresentado nesse trabalho. Hidrocarbonetos (46,84%), triterpenos (32,60%) e derivados de carotenoides (0,83%) foram considerados os constituintes majoritários de Hex-Le. Em contraste, hidrocarbonetos (41,89%), diterpenos (12,83%) e triterpenos (7,0%) foram encontrados em Hex-Sb. Quatro diterpenos (deidroabietano, sandaracopimaradieno, caur-16-eno e 13-metil-17-norcaur-15-eno) e um triterpeno (diplopteno) são relatados pela primeira vez no gênero *Cnidoscolus*.

**Palavras-chave:** plantas medicinais; Caatinga; terpenoides; *Cnidoscolus*.

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# **Chemical constituents of non-polar fractions obtained from *Cnidoscolus quercifolius* Pohl (Euphorbiaceae), a Brazilian medicinal plant native to Caatinga biome**

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## **1. Introduction**

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## 1. Introduction

Caatinga is an exclusively Brazilian biome, which occurs predominantly in the Northeastern Region and comprises approximately 10% of the Brazilian territory. This biome usually presents dry vegetation, characterized by semiarid climate, low and irregular rainfall and fertile soil.<sup>1,2</sup> Despite the adverse climatic conditions, Caatinga flora is very diverse and includes numerous species used as food source or in folk medicine. In a previous study, the use of 385 Caatinga species for medicinal purposes was verified. These species were distributed in about 265 genera and 91 different families.<sup>3</sup> Although the Caatinga biome presents several species with therapeutic potential, chemical and pharmacological investigations involving these plants are still scarce,<sup>4,5</sup> which makes this biome one of the least studied in the world.

*Cnidoscolus quercifolius* (syn. *C. phyllacanthus* (Mull. Arg.) Pax & L. Hoffm.) is a medicinal plant native to Caatinga, popularly known as favela, faveleira or urtiga-branca. In folk medicine, its leaves and stem barks are used to treat hemorrhoids, renal problems, ophthalmic diseases, injury, skin problems, urinary tract infection and inflammatory processes.<sup>3</sup> In fact, pharmacological investigations have demonstrated that extracts or isolated compounds from *C. quercifolius* have some therapeutic properties, such as antinociceptive,<sup>6</sup> anti-inflammatory,<sup>7</sup> antioxidant,<sup>8-10</sup> cytotoxic<sup>11</sup> and antimicrobial<sup>9</sup> activities.

The therapeutic properties of *C. quercifolius* are justified by the presence of bioactive secondary metabolites, mainly terpenoids. Phytochemical studies have resulted in the isolation of new tricyclic benzocyclohepten diterpenes, called favelins, a reference to the popular name of the species.<sup>12-14</sup> Endo et al.<sup>15</sup> also reported the isolation of favelanone and neofavelanone, new cyclopropane and cyclobutene tetracyclic derivatives, respectively. To date, these diterpenes have been reported exclusively in *C. quercifolius*, indicating that these compounds can be considered its chemotaxonomic markers. However, its chemical composition is still poorly known. In this paper, we describe the identification of compounds from leaves and stem-barks non-polar fractions of *C. quercifolius*, including compounds reported for the first time in the genus.

## 2. Experimental

### 2.1. Plant material

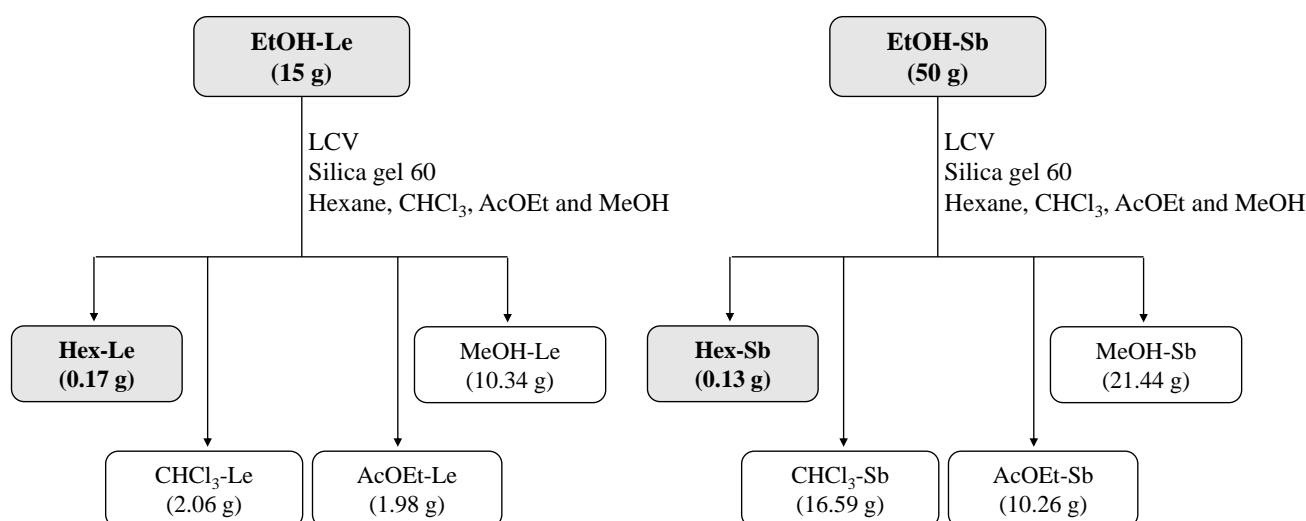
Leaves and stem-barks of *Cnidoscolus quercifolius* Pohl were collected in the city of Petrolina, in a Caatinga area (coordinates 09° 03' 55.30" S and 40° 20' 06.90" W), State of Pernambuco, Brazil, in February 2013. A voucher specimen (n° 19202) was deposited at the Herbário do Vale do São Francisco (HVASF), of the Universidade Federal do Vale do São Francisco (UNIVASF).

### 2.2. Chemicals

Homologous series of n-alkanes (C<sub>9</sub>H<sub>20</sub> – C<sub>40</sub>H<sub>82</sub>) were purchased from Merck<sup>®</sup> (Germany). All solvents (ethanol, hexane, chloroform, ethyl acetate and methanol) were purchased from Synth<sup>®</sup> (Brazil).

### 2.3. Extraction and fractionation

The dried and pulverized leaves (482 g) and stem-barks (1,481 g) of *C. quercifolius* were separately subjected to maceration with 95% ethanol for 72 h. After, the solution was filtered and concentrated under reduced pressure on a rotatory evaporator at 50 °C, producing 63 and 392 g of ethanol extract from leaves (EtOH-Le, 13.07%) and stem-barks (EtOH-Sb, 26.46%). Subsequently, an aliquot of EtOH-Le (15 g) and EtOH-Sb (50 g) was fractionated by liquid chromatography under vacuum (LCV), using silica gel 60 as stationary phase. Hexane (Hex), chloroform (CHCl<sub>3</sub>), ethyl acetate (AcOEt) and methanol (MeOH) were used as mobile phase, in increasing order of polarity, resulting in the respective fractions, as shown in figure 1. Hexane fractions (Hex-Le and Hex-Sb) were chosen for GC-MS analysis.



**Figure 1.** Fractionation of EtOH-Le and EtOH-Sb by liquid chromatography under vacuum (LCV). Hexane fractions (Hex-Le and Hex-Sb) were selected for GC-MS analysis.

### 2.4. Gas Chromatography - Mass Spectrometry (GC-MS) analysis

Qualitative and quantitative determination of the chemical constituents present in Hex-Le and Hex-Sb was performed by GC-MS, using a Shimadzu<sup>®</sup> gas chromatograph (QP-2010) interfaced with a mass spectrometer, employing the following chromatographic conditions: Phenomenex<sup>®</sup> ZB-5MS Zebron column (30.0 m x 0.25 mm x 0.25 mm); helium (99.999%) carrier gas at a constant flow of 1.1 ml.min<sup>-1</sup>; 1 µl injection volume; injector split ratio of 1:40; injector temperature 240 °C; electron impact mode at 70 eV; ion source temperature 280 °C. The oven temperature was programmed at 100 °C (isothermal for 5 min), with an increase

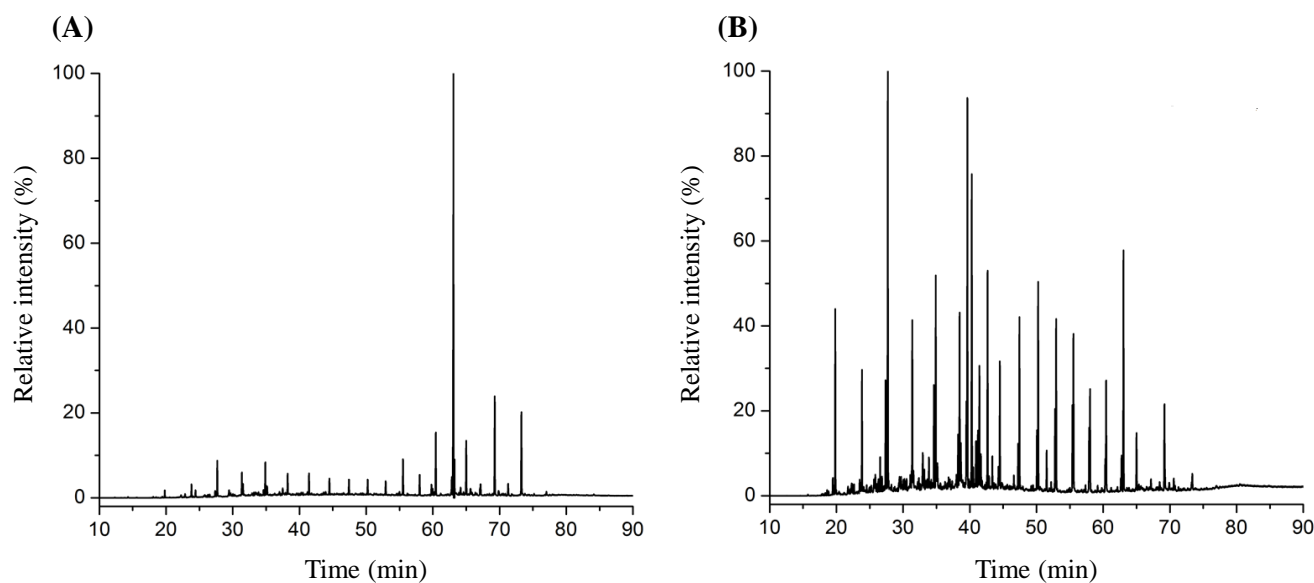
of 10 °C.min<sup>-1</sup> to 250 °C (isothermal for 5 min) and 10 °C.min<sup>-1</sup> to 280 °C (isothermal for 5 min). A mixture of linear hydrocarbons (C<sub>9</sub>H<sub>20</sub> – C<sub>40</sub>H<sub>82</sub>) was injected under the same experimental conditions.

## 2.5. Identification of compounds

Constituents were identified by comparison of their mass spectra with those of authentic compounds or with reference spectra in the computer library (Wiley7lib and NIST08lib). For some identified secondary metabolites (triterpenes, diterpenes and carotenoid derivatives), the fragmentation profile was proposed based on the peaks observed in the mass spectrum.

## 3. Results and Discussion

GC-MS chromatogram of Hex-Le revealed the presence of 49 peaks, of which 30 were identified, corresponding to 80.27% of its total chemical composition (Figure 2A). Among the identified compounds, 0.83% were carotenoid derivatives, 46.84% were long chain hydrocarbons or derivatives, and 32.60% corresponded to squalene, a triterpene considered the major constituent of the sample. Regarding Hex-Sb, the chromatogram showed 74 peaks (Figure 2B), of which 46 were identified, corresponding to 61.72% of its chemical composition. Most of the compounds were long chain hydrocarbons or derivatives (41.89%), followed by diterpenes (12.83%) and triterpenes (7.0%). Carotenoid derivatives were not identified in this sample. The major chemical constituents of Hex-Sb were hexadecane (7.48%) and sandaracopyramadiene (7.53%). All identified compounds are described in Table 1.



**Figure 2.** GC-MS chromatograms obtained for Hex-Le (A) and Hex-Sb.

**Table 1.** Chemical constituents of non-polar fractions from leaves (Hex-Le) and stem-bark (Hex-Sb) of *Cnidoscolus quercifolius*. Compounds were identified by CG-MS analysis

Phytochemical	Compound	Hex-Le		Hex-Sb	
		RT (min)	Content (%)	RT (min)	Content (%)
<i>Carotenoid derivatives</i>	$\beta$ -ionone	22.84	0.26	ND	ND
	Dihydroactinidiolide	24.41	0.57	ND	ND
	<b>Identified (%)</b>	-	<b>0.83</b>	-	<b>ND</b>
<i>Hydrocarbons and derivatives</i>	1-tridecene	ND	ND	19.45	0.28
	Tetradecane	19.80	0.56	19.82	3.00
	2,3,7-trimethyl-decane	ND	ND	22.27	0.12
	2-methyl-tetradecane	ND	ND	22.37	0.13
	3-methyl-tetradecane	ND	ND	22.65	0.13
	2-methyl-hexadecan-1-ol	ND	ND	23.48	0.21
	Pentadecane	23.81	0.99	23.82	1.99
	5-methyl-pentadecane	ND	ND	25.83	0.29
	2-methyl-pentadecane	ND	ND	26.29	0.24



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3-methyl-pentadecane	26.53	0.18	26.55	0.59
3-hexadecene	ND	ND	26.82	0.26
1-hexadecene	ND	ND	27.37	1.85
Hexadecane	27.67	2.93	27.70	7.48
3-hexyl-1,1,2-trimethyl-cyclobutane	ND	ND	27.84	0.15
2,6,10-trimethyl-pentadecane	29.45	0.56	29.45	0.15
1-cyclohexyl-decane	29.69	0.17	ND	ND
1-decyl-cyclopentane	ND	ND	29.70	0.19
2-methyl-hexadecane	ND	ND	30.03	0.17
2-methyl-heptadecane	ND	ND	30.29	0.11
2-phenyl-dodecane	ND	ND	31.07	0.26
Heptadecane	31.36	1.79	31.37	3.01
2,6,10,14-tetramethyl-pentadecane	31.52	1.40	31.51	0.50
7-methyl-hexadecane	ND	ND	32.94	0.75
4-ethyl-heptadecane	33.15	0.26	ND	ND
3-methyl-heptadecane	33.85	0.24	33.87	0.46

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1-octadecene	ND	ND	34.61	1.88
Octadecane	ND	ND	34.61	1.88
2,6,10,14-tetramethyl-hexadecane	35.11	1.15	ND	ND
4-cyclohexyl-tridecane	37.00	0.18	ND	ND
Hexadecan-1-ol	37.48	0.54	ND	ND
Nonadecane	38.22	1.69	38.24	0.91
Octasane	40.26	0.18	ND	ND
3-methyl-nonadecane	40.52	0.19	ND	ND
2-methyl-eicosane	ND	ND	40.53	0.36
1-tricosene	41.20	0.21	47.23	0.83
1-nonadecene	ND	ND	41.21	1.06
Eicosane	41.43	1.85	41.65	0.68
Octadecan-1-ol	43.91	0.17	ND	ND
Heneicosan-1-ol	ND	ND	44.28	0.32
Heneicosane	44.48	1.46	44.49	2.36
7-hexyl-eicosane	ND	ND	46.60	0.19

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	Docosane	47.41	1.34	47.43	3.22
	7-hexyl-docosane	ND	ND	52.19	0.14
	Pentacosane	60.43	5.69	55.35	2.96
	Nonacosane	55.52	3.09	ND	ND
	Hexacosane	62.83	1.79	58.02	1.09
	Heptacosan-1-ol	67.06	0.55	60.31	0.60
	Tetracontane	69.27	9.59	64.99	1.09
	Tetratetracontane	73.27	8.09	ND	ND
	<b>Identified (%)</b>	-	<b>46.84</b>	-	<b>41.89</b>
<i>Diterpenes</i>	Sandaracopimaradiene	ND	ND	39.63	7.53
	13-methyl-17-norkaur-15-ene	ND	ND	41.65	0.68
	Kaur-16-ene	ND	ND	42.66	4.38
	Dehydroabietane	ND	ND	42.89	0.24
	<b>Identified (%)</b>	-	<b>ND</b>	-	<b>12.83</b>
<i>Triterpenes</i>	Squalene	63.09	32.60	63.03	4.65
	Lupeol	ND	ND	69.18	2.15

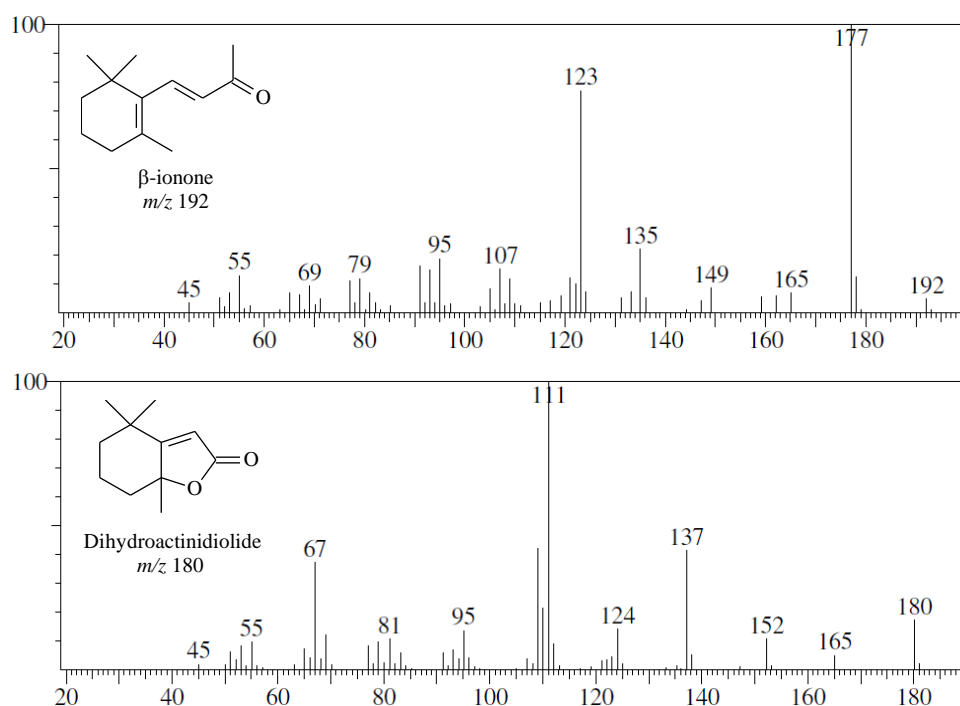
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Diploptene	ND	ND	73.35	0.20
<b>Identified (%)</b>	-	<b>32.60</b>	-	<b>7.00</b>
<b>Total identified (%)</b>		<b>80.27</b>		<b>61.72</b>

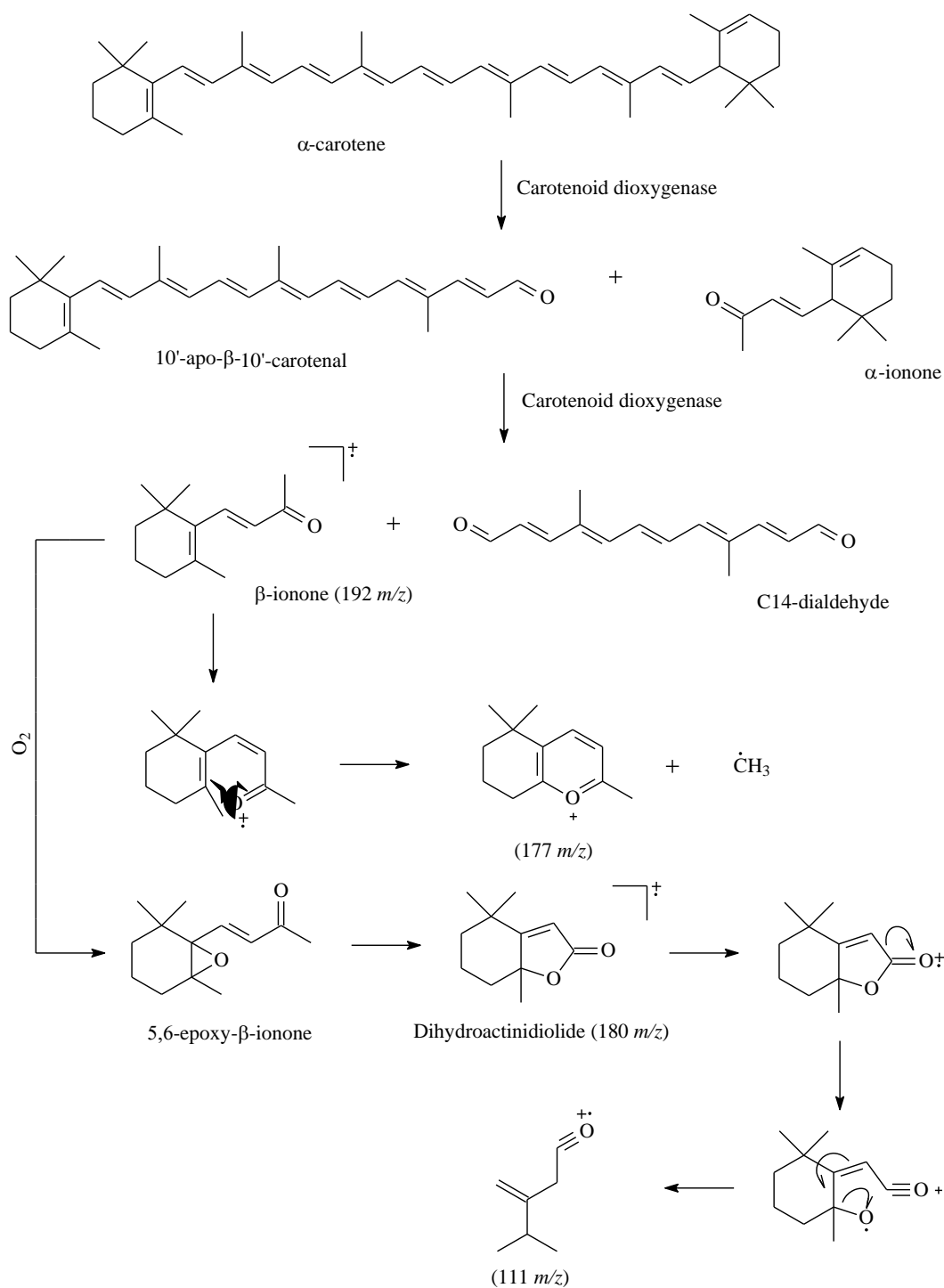
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RT: retention time. ND: compound not detected in sample.

Two carotenoid derivatives were identified in Hex-Le,  $\beta$ -ionone (0.26%) and dihydroactinidiolide (0.57%) (Figure 3). These constituents are considered products of carotenoid degradation through the action of carotenoid dioxygenase enzymes.<sup>16,17</sup> In fact, carotenoid degradation products indicate that the plant is in an oxidative stress condition, often associated with the presence of singlet oxygen ( $O_2^{\cdot}$ ).<sup>18,19</sup> A summary of the biosynthesis as well as the proposed fragmentation profile for  $\beta$ -ionone and dihydroactinidiolide are shown in figure 4. Initially, an  $\alpha$ -carotene molecule is used by the carotenoid dioxygenase enzyme as substrate, producing  $\alpha$ -ionone and 10'-apo- $\beta$ -10'-carotenal, which is then converted to  $\beta$ -ionone ( $m/z$  192). In the mass spectra,  $\beta$ -ionone loses a methyl group by a cleavage adjacent to the annular double bond, yielding a new fragment ( $m/z$  177), compatible with the base peak (Figure 3). In an oxidative stress situation,  $\beta$ -ionone molecule is readily oxidized to 5,6-epoxy- $\beta$ -ionone and, after a rearrangement step, leads to the formation of dihydroactinidiolide ( $m/z$  180).<sup>16,17</sup> Rupture of the lactone ring of this molecule releases a stable fragment ( $m/z$  111), compatible with the base peak observed in the mass spectra (Figure 3).



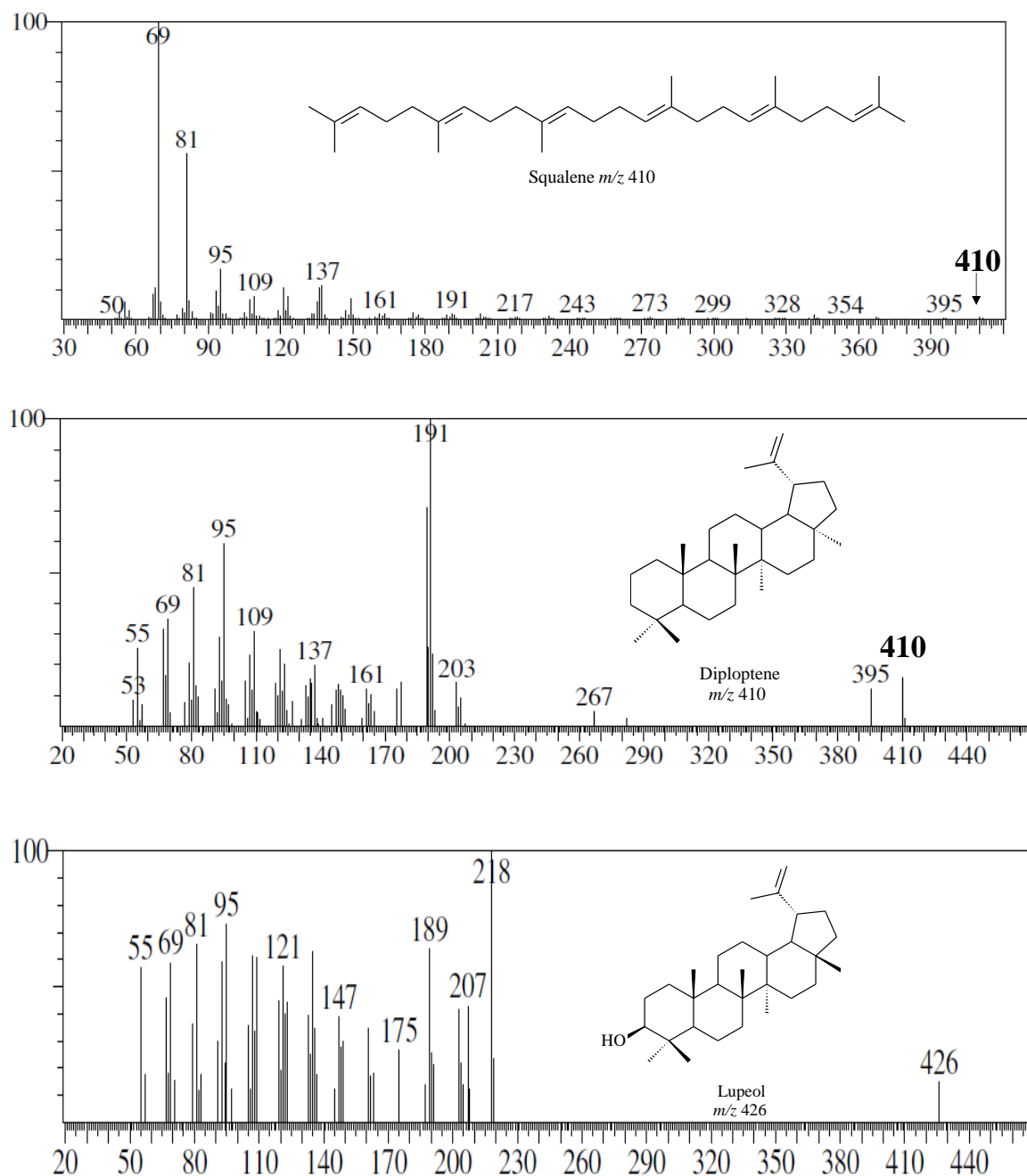
**Figure 3.** Mass spectrum obtained for  $\beta$ -ionone and dihydroactinidiolide identified in Hex-Le.



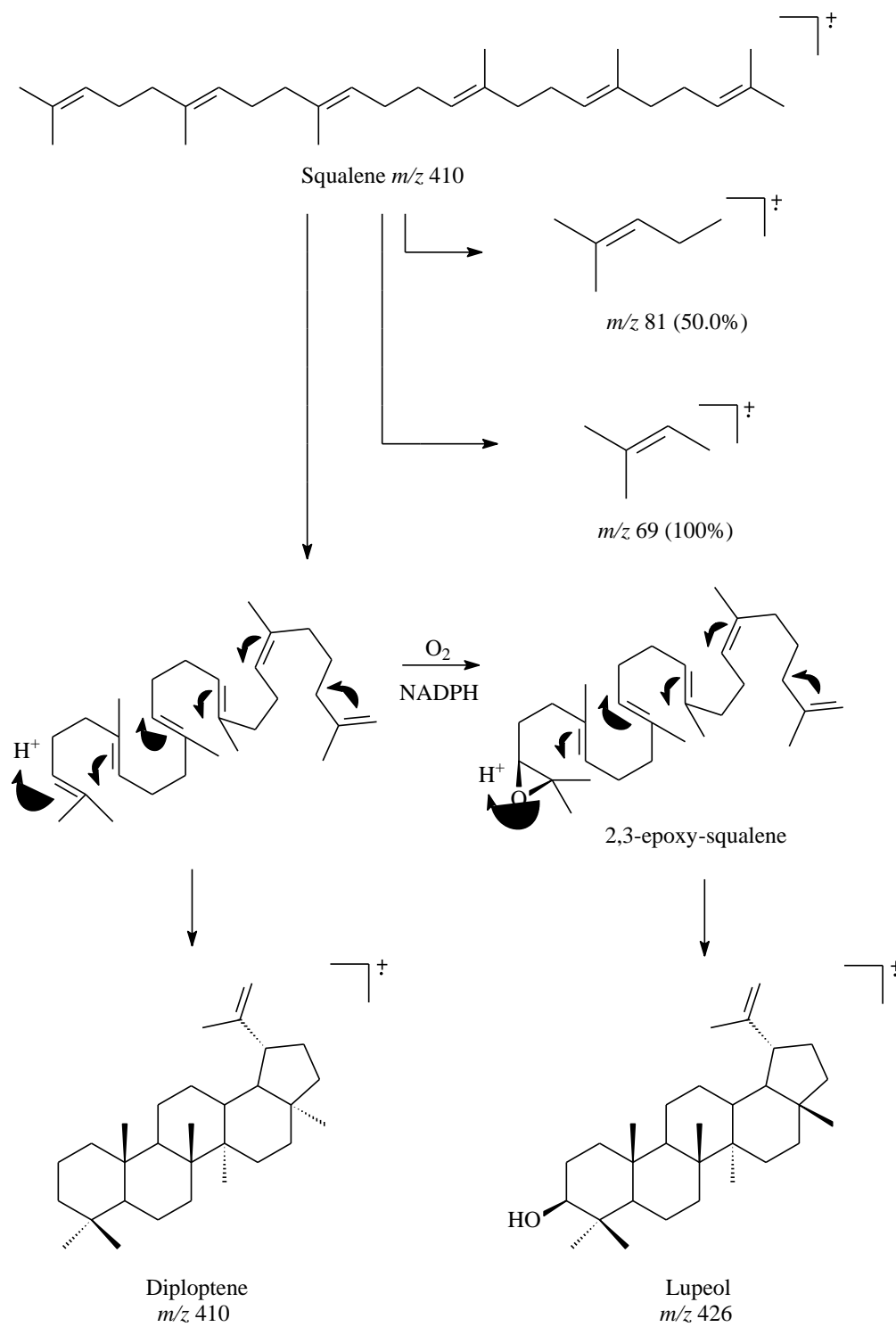
**Figure 4.** Biosynthesis and proposed fragmentation of carotenoid derivatives  $\beta$ -ionone and dihydroactinidiolide.

The triterpene squalene was found in lower amount in Hex-Sb (4.65%). However, other triterpenes were identified in this sample (diploptene 0.20%, lupeol 2.15%) and its mass spectra are shown in Figure 5. Squalene is considered the precursor of diploptene and lupeol, as shown in Figure 6. In its mass spectrum,  $m/z$  69 (base peak) and 81 were the most stable fragments, already described in the literature as

characteristic fragments of this molecule.<sup>20</sup> For lupeol and diploptene, spectral data contributed significantly to the characterization of these molecules, such as molecular ion ( $m/z$  426 and  $m/z$  410, respectively) and base peaks ( $m/z$  191 and  $m/z$  218, respectively).<sup>21,22</sup> Besides these, other fragments are shown in figure 7. Paula et al.<sup>14</sup> have reported the presence of lupeol and derivatives in *C. quercifolius* extracts. However, there are no reports of the presence of diploptene in *Cnidioscolus* species to date.

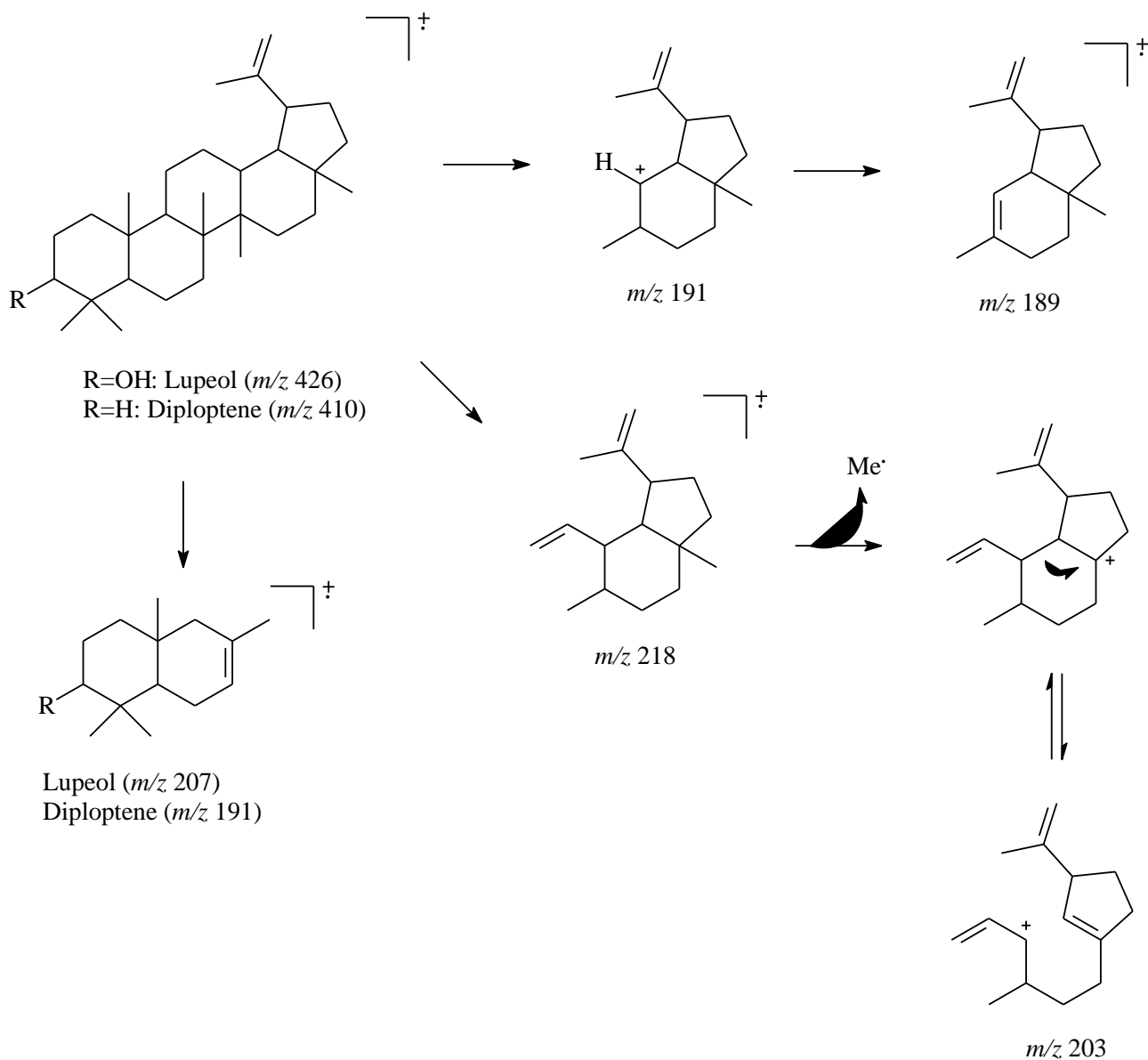


**Figure 5.** Mass spectra obtained for the triterpenes identified in Hex-Sb.



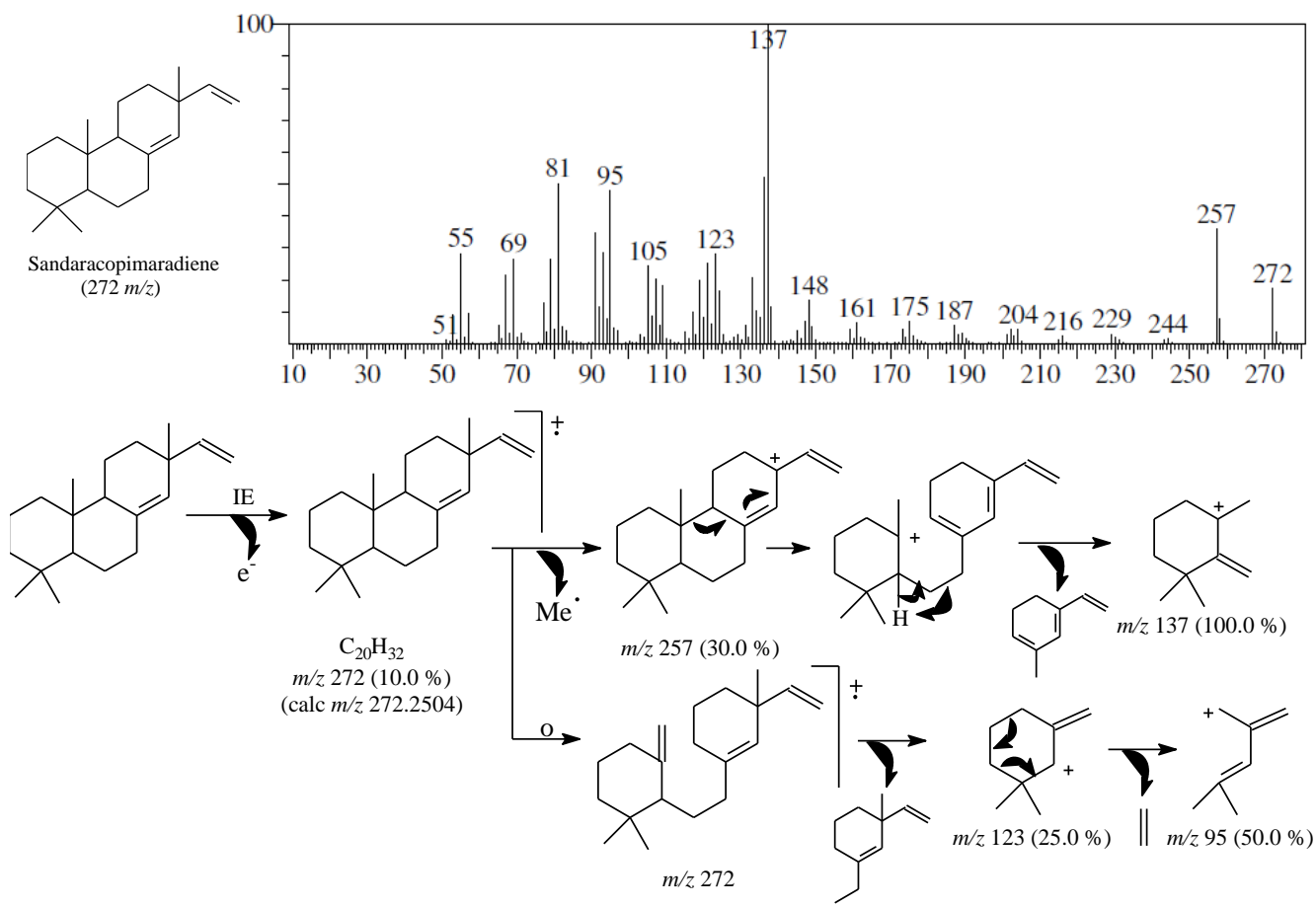
**Figure 6.** Simplified biosynthetic route for diploptene and lupeol, and proposed fragmentation profile for squalene.





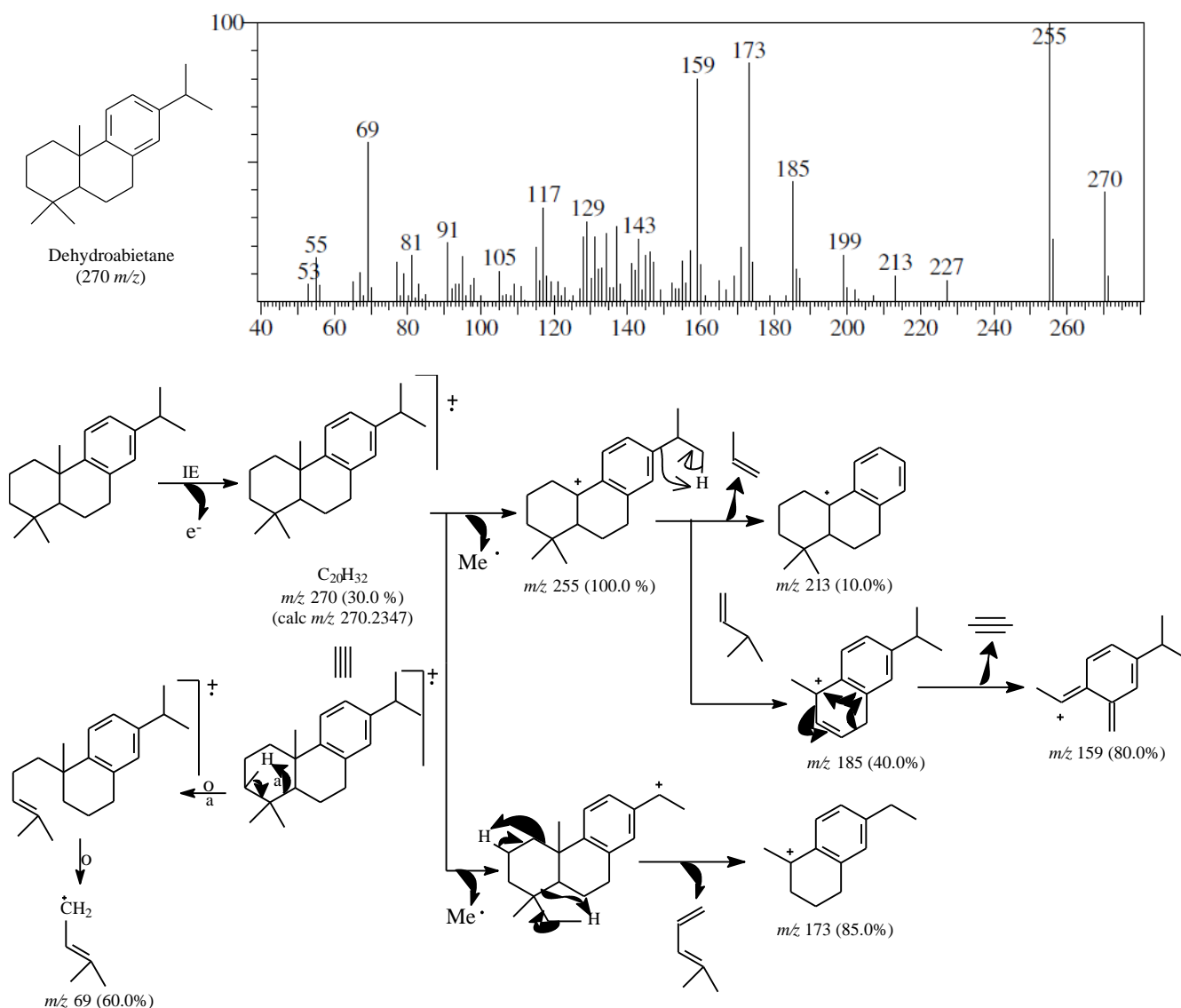
**Figure 7.** Proposed fragmentation of lupeol and diploptene, identified in Hex-Sb.

GC-MS analysis also showed abietane (dehydroabietane 0.24%), pimarane (sandaracopimaradiene 7.53%) and kaurene (kaur-16-ene 4.38%, 13-methyl-17-norkaur-15-ene 0.68%) diterpenes in Hex-Sb, reported for the first time in *Cnidocolus*. Figure 8 shows the mass spectra of sandaracopimaradiene as well as the proposed fragmentation profile for the molecule. An abundant fragment ( $m/z$  257) was registered after loss of the methyl group, while the base peak ( $m/z$  137) was originated after a B-ring cleavage, as shown in the figure.



**Figure 8.** Mass spectra and fragmentation proposed for sandaracopimaradiene, identified in Hex-Sb.

Figure 9 shows dehydroabietane mass spectra and its fragmentation profile. Initially, the molecule loses a methyl group, yielding a stable fragment, compatible with the base peak ( $m/z$  255). From that fragment, one isopropyl group is removed, affording another fragment ( $m/z$  213). In addition, A-ring cleavage has been proposed, yielding a fragment ( $m/z$  185) which is then further cleaved, resulting in a new fragment ( $m/z$  159).

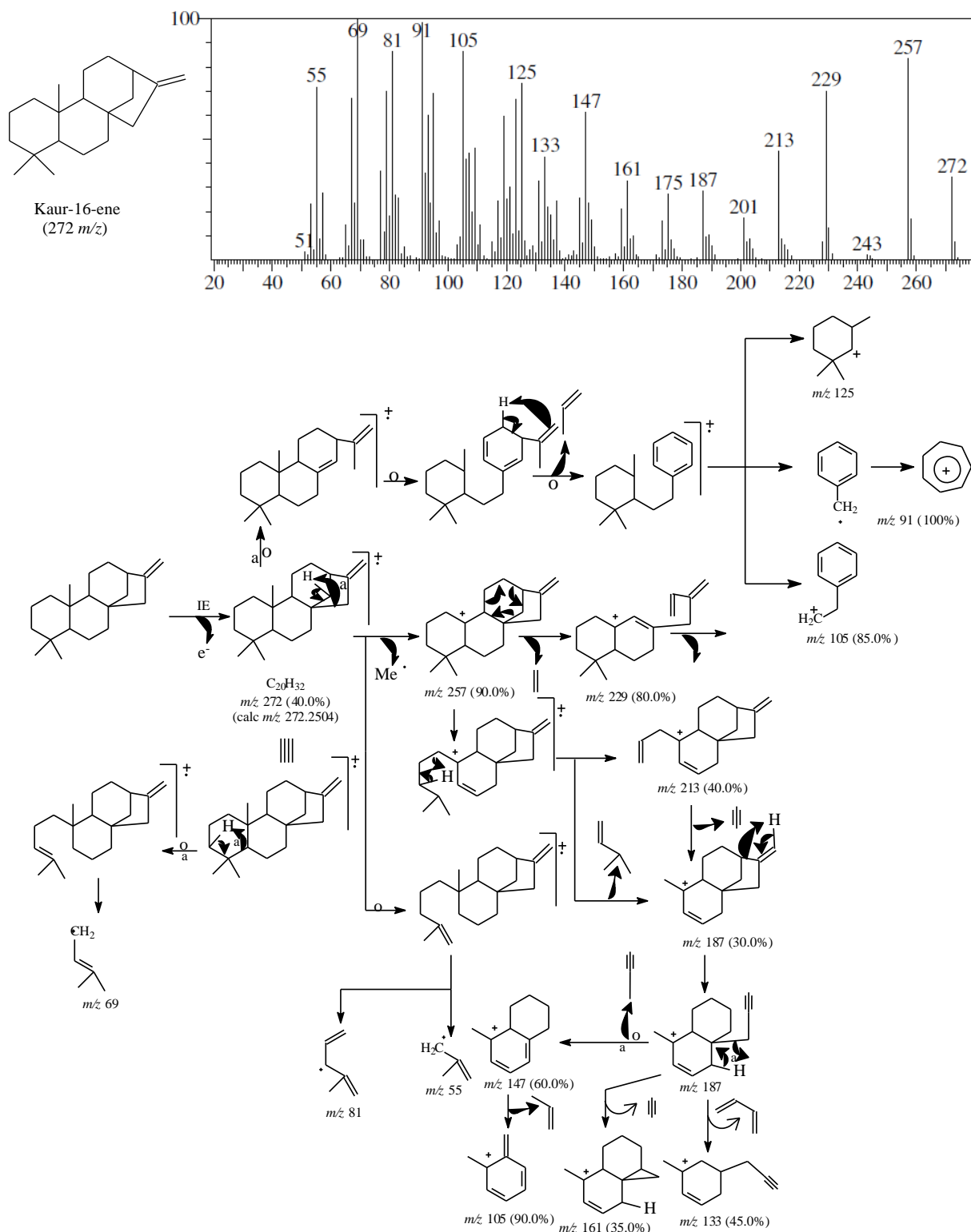


**Figure 9.** Mass spectra and fragmentation proposed for dehydroabietane, identified in Hex-Sb.

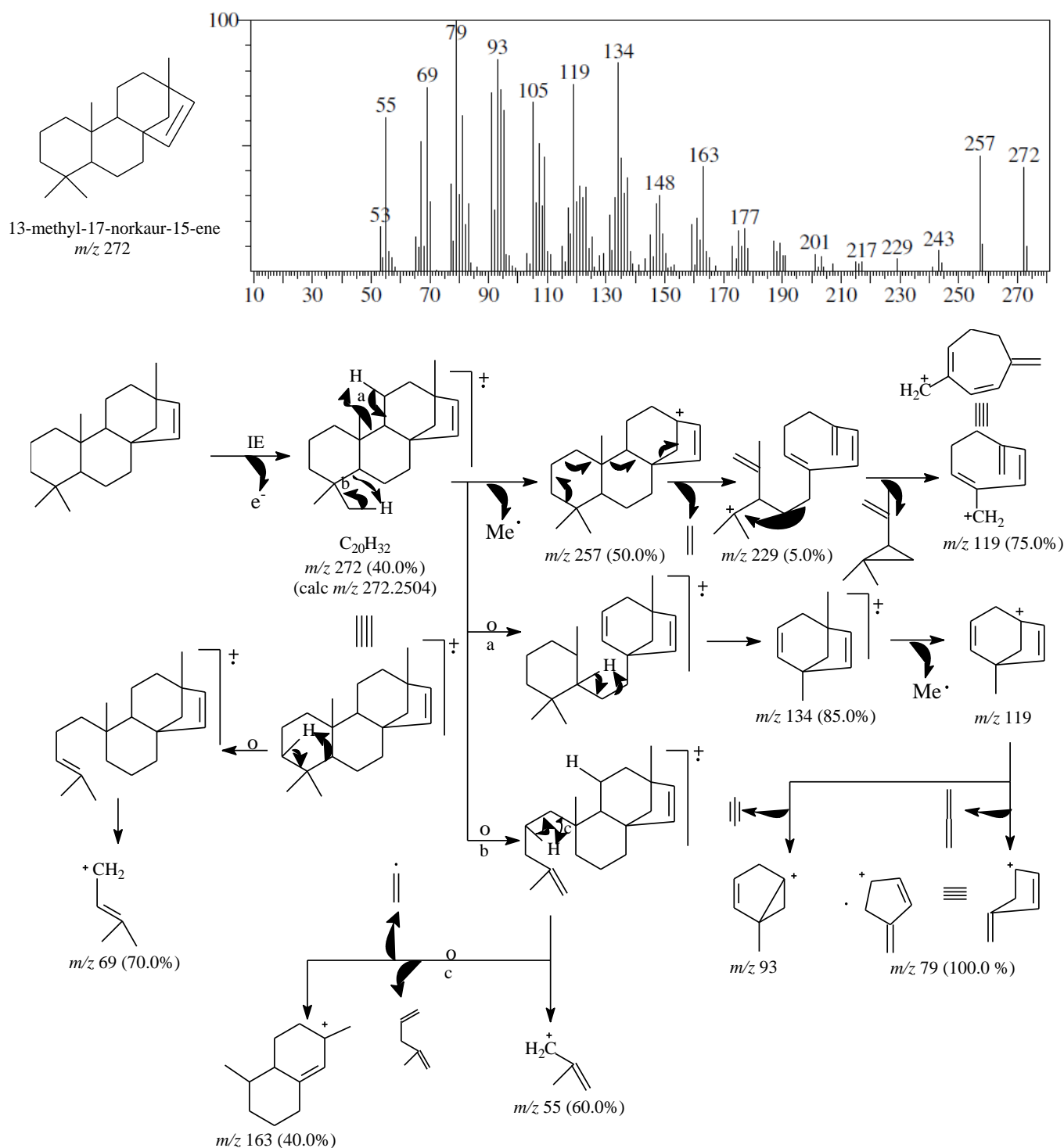
In the mass spectra of kaur-16-ene, it was observed a molecular ion peak ( $m/z$  272), compatible with the molecular formula  $C_{20}H_{32}$ . After ionization, this diterpene also loses a methyl group, providing a stable fragment ( $m/z$  257). Subsequently, C-ring undergoes a retro Diels-Alder cleavage, yielding a fragment in  $m/z$  229. Hydrogen rearrangements were proposed in the fragmentation profile, resulting in new fragments ( $m/z$  187, 147, 213 and 133), as shown in the figure 10. In addition, D-ring hydrogen rearrangements led to the formation of a stable fragment, attributed to the tropylium ion ( $m/z$  91), compatible with the base peak found in the mass spectrum.

For 13-methyl-17-norkaur-15-ene, fragmentation begins with the loss of C-13 methyl, generating a relatively stable fragment ( $m/z$  257). This fragment undergoes a series of rearrangements, resulting in  $m/z$  229 and  $m/z$  119 peaks. The  $m/z$  257 fragment undergoes a B-ring cleavage and then rearrangement of hydrogens, releasing a new fragment in  $m/z$  134. This fragment loses a methyl group, resulting in the  $m/z$

119 peak. Formation of the fragment corresponding to the base peak of the spectrum ( $m/z$  79) occurs by cleavage of the last fragment recorded in  $m/z$  119, as shown in figure 11. The complete fragmentation profile of the diterpenes identified in Hex-Sb is shown in the figures 8-11.



**Figure 10.** Mass spectra and fragmentation proposed for kaur-16-ene, identified in Hex-Sb.



**Figure 11.** Mass spectra and fragmentation proposed for 13-methyl-17-norkaur-15-ene, identified in Hex-Sb.

#### 4. Conclusion

In summary, this study reported a chemical investigation of non-polar fractions obtained from leaves and stem-barks of *C. quercifolius*. We describe for the first time the identification of diterpenes dehydroabietane, sandaracopimaradiene, kaur-16-ene and 13-methyl-17-norkaur-15-ene, in a *Cnidoscopus*

species. Indicators of oxidative stress were also identified ( $\beta$ -ionone and dihydroactinidiolide), in addition to triterpenes (lupeol and diploptene) commonly found in plant species. In this sense, our investigation has contributed to the chemical knowledge of *C. quercifolius*, a Brazilian medicinal plant from Caatinga biome.

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