SUPPLEMENTARY MATERIAL

Two new eudesman-4α-ol epoxides from the stem essential oil of *Laggera pterodonta* from Côte d'Ivoire

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The investigation of the stem essential oil of *Laggera pterodonta* (DC.) Sch. Bip. ex Oliv. (Asteraceae) from Côte d'Ivoire was carried out, using a combination of chromatographic (GC-RI, CC, pc-GC) and spectroscopic (GC-MS, ^{13}C NMR) techniques. This study led to the identification of fifty constituents of which two new natural compounds namely 7β,11β-epoxy-eudesman-4α-ol and 7α,11α-epoxy-eudesman-4α-ol. Their structures were elucidated by 1D and 2D NMR spectroscopy after pc-GC purifying. Finaly 98.9% of the whole composition of the oil was identified with a high amount of 2,5-dimethoxy-*p*-cymene (78.9%). The other significant components were α-humulene (6.2%), (*E*)-β-caryophyllene (1.7%), thymyl methyl oxide (1.7%), α-phellandrene (1.5%), *p*-cymene (1.2%), (3αH,4βH,6αH,1αMe)-1,6-epoxy-3-hydroxycarvotanacetone angelic acid ester (1.1%) and 10-*epi*-γ-eudesmol (1.0%).

Keywords: *Laggera pterodonta*; stem oil; 2,5-dimethoxy-*p*-cymene; eudesman-4α-ol epoxide; Côte d'Ivoire.

Experimental

Plant material and isolation procedure

The plant material was harvested in Marabadiassa, (Region of Vallée du Bandama, Department of Béoumi, Central Côte d'Ivoire) in September 2016 and authenticated by Mr Jean Assi, technician at the Herbarium of the Centre National de Floristique (Abidjan, Côte d'Ivoire) and Mr Henry Téré from the Centre Suisse de Recherche (Abidjan, Côte d'Ivoire). A voucher specimen has been deposited at the herbarium of the Centre National de Floristique (CNF), Abidjan, with the reference LAA 14631. The essential oil was obtained in 0.040% (w/w) yield, by successive hydrodistillations of a total of 14250.2g of fresh stems. Each hydrodistillation was performed using a Clevenger-type apparatus for 3 hours.

Essential oil fractionation

The essential oil (5.702g) was chromatographed on silica gel (200-500µm, 150g) and six fractions were first eluted, using a gradient of pentane:diethyl ether (P:DE) from 100:0 to 0:100. Fraction F1 (656mg, eluted with P) contained hydrocarbons; fractions F2-F5 (4439, 244, 119 and 69mg, respectively, eluted with P:DE mixtures) contained medium polar compounds; fraction F6 (90mg, eluted with DE) contained polar compounds. Fraction F5 was fractionated on silica gel (35-70µm, 4g) with P:DE = 95:5 to 0:100 and yielded F5.1 to F5.4 (3, 36, 27 and 1mg, respectively). Fraction F5.3 was subjected to silica gel chromatography (35-70µm, 1.5g) and yielded sub-fractions F5.3.1 (21 mg; P:DE = 90:10) and F5.3.2 (3mg; P:DE = 80:20). Sub-fraction F5.3.1 was finally submitted to Preparative Capillary-Gas Chromatography in order to purify compounds **48** and **49**.

Gas chromatography with FID associated to RI

Analyses were carried out using a Clarus 500 Perkin Elmer (Perkin Elmer, Courtaboeuf, France) system equipped with a FID and two fused-silica capillary columns (50m x 0.22 mm, film thickness 0.25 µm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min; injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: helium (0.8 mL/min); split: 1/60; injected volume: 0.5 mL. The relative proportions of the oil constituents were expressed as percentages obtained by peak-area normalization, without using correcting factors. Retention indices (RI) were determined relative to the retention times of a series of n-alkanes with linear interpolation (Target Compounds software from Perkin Elmer).

Gas chromatography-mass spectrometry in electron impact mode

Original sample and all fractions were analysed with a Clarus SQ8S Perkin Elmer TurboMass detector (quadrupole), directly coupled to a Clarus 580 Perkin-Elmer Autosystem XL, equipped with a Rtx-1 (polydimethylsiloxane) fused-silica capillary column (60 m 9 0.22 mm i.d., film thickness 0.25 µm). The oven temp. was programmed rising from 60 to 230 °C at 2°/min and then held isothermal at 230°

for 45 min; injector temp., 250 °C; ion-source temp.,150 °C; carrier gas, He (1 ml/min); split ratio, 1:80; injection volume, 0.2 ml; ionization energy, 70 eV. The electron ionization (EI) mass spectra were acquired over the mass range 35 – 350 Da.

Preparative capillary-gas chromatography

Isolations of 7β,11β-epoxy-eudesman-4α-ol **48** and 7α,11α-epoxy-eudesman-4α-ol **49** were performed using an Agilent 6890 Plus gas chromatograph coupled to a Gerstel preparative fraction collector (PFC) (Agilent, Santa Clara, CA, USA), operated under Chemstation Rev A.10.02/Gerstel Maestro 1.3.8.14. The GC was equipped with a Phenomenex ZB-5 megabore capillary column (30 m × 0.53 mm; 3.0 µm film thick.). A Graphpack effluent splitter was connected to the column outlet, and additionally mounted with 0.1 mm and 0.32 mm deactivated fused-silica capillary restrictors (1 m each) to provide an FID/PFC ratio of ~1/9. The transfer line and the PFC were maintained at 230 °C. The injected volume is 1 µL in splitless mode. The oven temperature was ramped from 70 to 120 °C at 10 °C/min, then from 120°C to 250°C at 20°C/min. The system was operated in constant pressure mode at 35 kPa (Carrier gas H_2). Compound was trapped at 5-10 °C in Gerstel U-type glass tubes by programming cutting times into the operating software allowing accurate automated operation. The isolation of any unknown compound in amounts sufficient for NMR analysis required 150-400 GC runs, and to avoid all contaminations, the product was collected directly in a NMR tube.

Gas chromatography-high resolution mass spectrometry

High-resolution EI-mass spectra were recorded using an Agilent 7200 GC-QTOF system, equipped with a Agilent J&W, VF-waxMS capillary column (30 m × 0.25 mm; 0.25 µm film thick). The mass spectrometer was operated at 70 eV with an acquisition rate of 2 GHz over a 35−450 m/z range, affording a resolution of ~8000. Injection volume 1 µL; split ratio 1:20; inlet temperature 250 °C, detector temperature 230 °C; column flow (He) 1.2 mL/min; temperature program for oven 60 °C (5 min isotherm) to 240 °C at 5 °C/min (10 min final isotherm).

Nuclear magnetic resonance

Essential oil and fractions nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 400.132 MHz for ^{1}H and 100.63 MHz for ^{13}C, equipped with a 5 mm probe, in $CDCl_3$, with all shifts referred to internal TMS. The ^{1}H NMR spectra were recorded with the following parameters: pulse width (PW), 4.3 µs; relaxation delay 1 s and acquisition time 2.6 s for 32 K data table with a spectral width (SW) of 6000 Hz. ^{13}C NMR spectra of the oil samples were recorded with the following parameters: pulse width = 4 µs (flip angle 45°); acquisition time = 2.7 s for 128K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. Standard pulse sequences from Bruker library were used for two-dimensional spectra. Gradient-enhanced sequences were used for the heteronuclear two dimensional experiments.

NMR spectra of 7β,11β-epoxy-eudesman-4α-ol **48** and 7α,11α-epoxy-eudesman-4α-ol **49** were recorded in C_6D_6 at 298 K on a Bruker Avance DRX 500 spectrometer operating at 500.13 MHz for ^1H and 125.75 MHz for ^{13}C. In order to increase sensitivity, ^{13}C NMR spectra such as broadband-^{13}C, DEPT 135 and DEPT 90 were run with a direct probe head (5 mm PADUL ^{13}C–^1H Z-GRD). 1D- and 2D NMR spectra such as ^1H, COSY, NOESY, HSQC, HMBC were run with an inverse probe head (5 mm PHTXI ^1H–^{13}C/^{15}N ZGRD). Spectrum calibration was performed by using the C_6D_6 signal as internal reference (7.16 ppm for ^1H NMR, 128.06 ppm for ^{13}C NMR). Chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants (*J*) in hertz. All NMR experiments were carried out using pulse sequences supplied by the spectrometer manufacturer (Bruker Topspin™) and processed via Mestrelab MestreNOVA software (Version 6.0.2-5475).

Identification of individual components

Identification of the individual components was carried out: (i) by comparison of their GC retention indices (RI) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation with those of reference compounds (König et al. 2001); (ii) on computer matching against commercial mass spectral libraries (National Institute of Standards and Technology. 1999; König et al. 2001; Adams 2007); (iii) on comparison of the signals in the ^{13}C NMR spectra of the mixtures with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi et al. 1995; Kambiré et al. 2018). This method allows the identification of individual components of the essential oil at content as low as 0.4-0.5%. A few compounds were identified by comparison with literature data.

References

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Table S1. Chemical composition of *Laggera pterodonta* stem essential oil

	Compounds [a]	RI1[b]	Rip[b]	%	Identification
1	α-Pinene	930	1016	tr	RI, MS
2	Sabinene	965	1123	0.3	RI, MS, *^{13}C NMR*
3	Myrcene	980	1161	0.1	RI, MS, *^{13}C NMR*
4	α-Phellandrene	996	1166	**1.5**	RI, MS, ^{13}C NMR
5	α-Terpinene	1008	1181	tr	RI, MS
6	*p*-Cymene	1011	1271	**1.2**	RI, MS, ^{13}C NMR
7	Limonene	1020	1202	0.7	RI, MS, ^{13}C NMR
8	1,8-Cineole*	1020	1209	tr	RI, MS
9	γ-Terpinene	1048	1244	tr	RI, MS
10	*trans*- *p*-Menth-2-en-1-ol	1106	1621	0.1	RI, MS, *^{13}C NMR*
11	*cis*- *p*-Menth-2-en-1-ol	1121	1638	0.1	RI, MS, *^{13}C NMR*
12	Albene	1151	1316	0.3	RI, MS, *^{13}C NMR*
13	Terpinen-4-ol	1160	1601	0.1	RI, MS, *^{13}C NMR*
14	Thymyl methyle oxide	1213	1593	**1.7**	RI, MS, ^{13}C NMR
15	Carvacryl methyle oxide	1224	1601	0.2	RI, MS, *^{13}C NMR*
16	Geraniol	1232	1843	tr	RI, MS
17	Geranial	1242	1734	0.1	RI, MS
18	Thymol	1267	2179	0.2	RI, MS, *^{13}C NMR*
19	Silphinene	1343	1466	tr	RI, MS
20	Geranyl acetate	1358	1753	tr	RI, MS, *^{13}C NMR*
21	β-Elemene	1385	1584	0.1	RI, MS, *^{13}C NMR*
22	2,5-Dimethoxy-*p*-cymene	1400	1869	**78.9**	RI, MS, ^{13}C NMR
23	*(E)*-β-Caryophyllene	1416	1593	**1.7**	RI, MS, ^{13}C NMR
24	γ-Elemene	1426	1632	0.1	RI, MS, *^{13}C NMR*
25	*epi*-β-Santalene	1441	1629	0.1	RI, MS, *^{13}C NMR*
26	α-Humulene	1449	1665	**6.2**	RI, MS, ^{13}C NMR
27	γ-Muurolene	1471	1675	tr	RI, MS
28	Germacrene D	1474	1704	0.1	RI, MS, *^{13}C NMR*
29	γ-Humulene	1476	1714	0.2	RI, MS, *^{13}C NMR*
30	β-Selinene	1479	1683	0.2	RI, MS, *^{13}C NMR*
31	Bicyclogermacrene	1488	1737	0.1	RI, MS, *^{13}C NMR*
32	*trans*-Dihydroagarofurane	1493	1715	0.2	RI, MS, *^{13}C NMR*
33	δ-Cadinene	1512	1748	0.1	RI, MS, *^{13}C NMR*
34	β-Elemol	1531	2077	0.1	RI, MS, *^{13}C NMR*
35	neryl isovalerate	1563	1876	0.2	RI, MS, *^{13}C NMR*
36	Caryophyllene oxide	1567	1977	0.2	RI, MS, *^{13}C NMR*
37	Rosifoliol	1586	2094	0.1	RI, MS, *^{13}C NMR*
38	Humulene oxide II	1591	2033	0.5	RI, MS, ^{13}C NMR
39	Viridiflorol	1603	2103	tr	RI, MS
40	10-*epi*-γ-Eudesmol	1606	2098	**1.0**	RI, MS, ^{13}C NMR
41	τ-Muurolol	1623	2177	0.1	RI, MS, *^{13}C NMR*
42	τ-Cadinol	1626	2165	0.2	RI, MS, *^{13}C NMR*
43	α-Eudesmol	1628	2220	0.1	RI, MS, *^{13}C NMR*
44	β-Eudesmol	1632	2235	tr	RI, MS
45	Intermedeol	1641	2225	0.1	RI, MS, *^{13}C NMR*
46	7-*epi*-α-Eudesmol	1642	2220	0.1	RI, MS, *^{13}C NMR*
47	Eudesm-7(11)-en-4α-ol	1676	2290	0.4	RI, MS, ^{13}C NMR
48	**7β,11β-Epoxy-eudesman-4α-ol**	1731	2530	0.1	QTOF-MS, 1D, 2D NMR
49	**7α,11α-Epoxy-eudesman-4α-ol**	1750	2549	0.3	QTOF-MS, 1D, 2D NMR
50	(3αH,4βH,6αH,1αMe)-1,6-Epoxy-3-hydroxycarvotanacetone, angelic acid ester	1767	2517	**1.1**	RI, MS, ^{13}C NMR

Monoterpene hydrocarbons	4.0
Oxygenated monoterpenes	81.4
Sesquiterpene hydrocarbons	8.8
Oxygenated sesquiterpenes	4.7
Total identified	**98.9**

[a]Order of elution and percentages were given on an apolar column (BP1), except components with an asterisk (*), which percentages were taken on polar column (BP20)

[b]RI1, Rip = Retention indices measured on apolar and polar capillary column respectively. tr = traces level (<0.05%).

All compounds were identified by GC(RI) and GC-MS. ^{13}C NMR, components were identified by NMR in the essential oil and obvious in at least one fraction of chromatography, *^{13}C NMR*, components were identified by NMR in one fraction of chromatography. Compounds **48** and **49** are new natural stereoisomers.

Table S2. NMR data of 7β,11β-epoxy-eudesman-4α-ol **48** and 7α,11α-epoxy-eudesman-4α-ol **49**

C	δC (ppm)		DEPT	δH (ppm) & Multiplicity (J,Hz)				HMBC	COSY	NOESY	
	48	**49**		**48**		**49**				**48**	**49**
1	40.88	41.01	CH2	1.24	m_c	1.24	m_c	2, 3, 9, 10	2.3	2, 3, 5, 9	2, 3, 5, 9
				0.89	m_c	0.97	m_c	2, 3, 4, 10	2,3	2, 3, 14, 15	2, 3, 14, 15
2	20.48	20.60	CH2	1.34	m_c	1.34	m_c	1, 3, 4, 5, 10	1,3	1, 3, 14,15	1, 3, 14, 15
3	44.06	44.03	CH2	1.26	m_c	1.66	m_c	4, 1, 15, 2, 5	2,1	1, 2, 5	1, 2, 5
				1.06	m_c	1.04	m_c	1, 4, 2, 15	2.1	1, 2, 14, 15	1, 2, 14, 15
4	71.19	71.20	Cq	-	-	-	-	-	-	-	-
5	54.43	52.16	CH	1.16	dd (13.1 ; 2.5)	1.54	m_c	4, 6, 9, 10, 14, 15	6	3, 6, **12, 13**	3, 6
6	26.89	25.60	CH2	1.93	dt (13.0 ; 2.5)	1.90	dt (13.3 ; 2.6)	10, 8, 7	5	8, 14, 15	8, 12, 14, 15
				1.59	t (13.0)	1.40	t (13.3)	10, 8, 11	5	8, 12	8, 12
7	61.50	62.16	Cq	-	-	-	-	-	-	-	-
8	27.53	26.61	CH2	1.86	dd (14.0 ; 3.9)	1.58	m_c	9, 7, 10	9	6, 9	6, 9
				1.49	dq(13.7 ; 3.2)	1.33	m_c	9, 1, 10, 7	9	6, 9, 13	6, 9, 13
9	43.17	42.34	CH2	1.55	m_c	1.57	m_c	5, 7, 11	8	5, 8, 14	5, 8
				1.18	t (12.5)	1.24	m_c	5, 7, 11	8	1, 8	1, 8, 14
10	34.45	34.41	Cq	-	-	-	-	-	-	-	-
11	66.94	65.75	Cq	-	-	-	-	-	-	-	-
12	20.98	20.70	CH3	1.24	s	1.19	s	7, 11, 12	-	**5**, 6, 13	13, **14, 15**
13	21.19	20.89	CH3	1.38	s	1.26	s	7, 11, 13	-	**5**, 8, 12	12, 13, **14, 15**
14	18.21	17.94	CH3	0.72	s	0.73	s	10, 1, 5	-	1, 2, 3, 6, 9, **15**	1, 2, 3, 6, **12**, **13**, **15**
15	22.65	21.90	CH3	0.86	s	1.00	s	3, 4, 5	-	1, 2, 3, 6, **14**	1, 2, 3, 6, **12, 13**, **14**

DEPT, Distortionless Enhancement by Polarization Transfer; HMBC, Heteronuclear Multiple Bond Correlation;
COSY, COrrelation SpectroscopY; NOESY, Nuclear Overhauser Effect SpectroscopY.
s = singlet, d = doublet, t = triplet, q = quadruplet, m_c = complex multiplet.

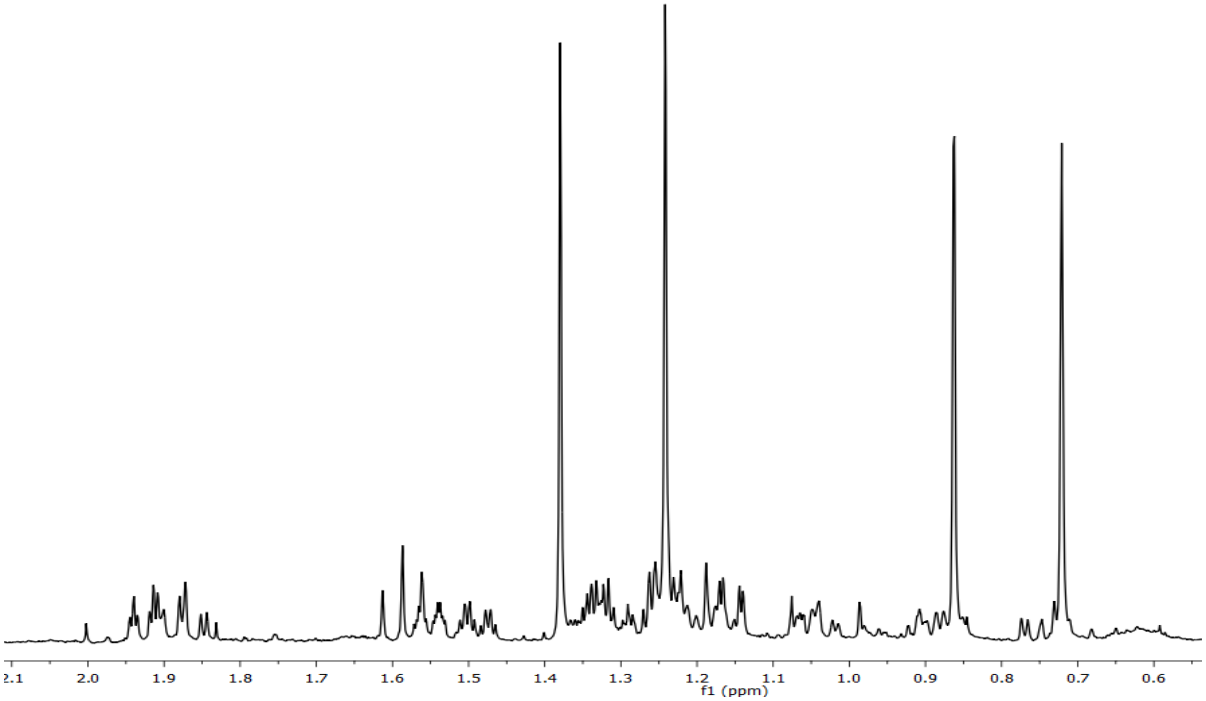

Figure S1. ^{1}H NMR spectrum of 7β,11β-epoxy-eudesman-4α-ol (**48)**

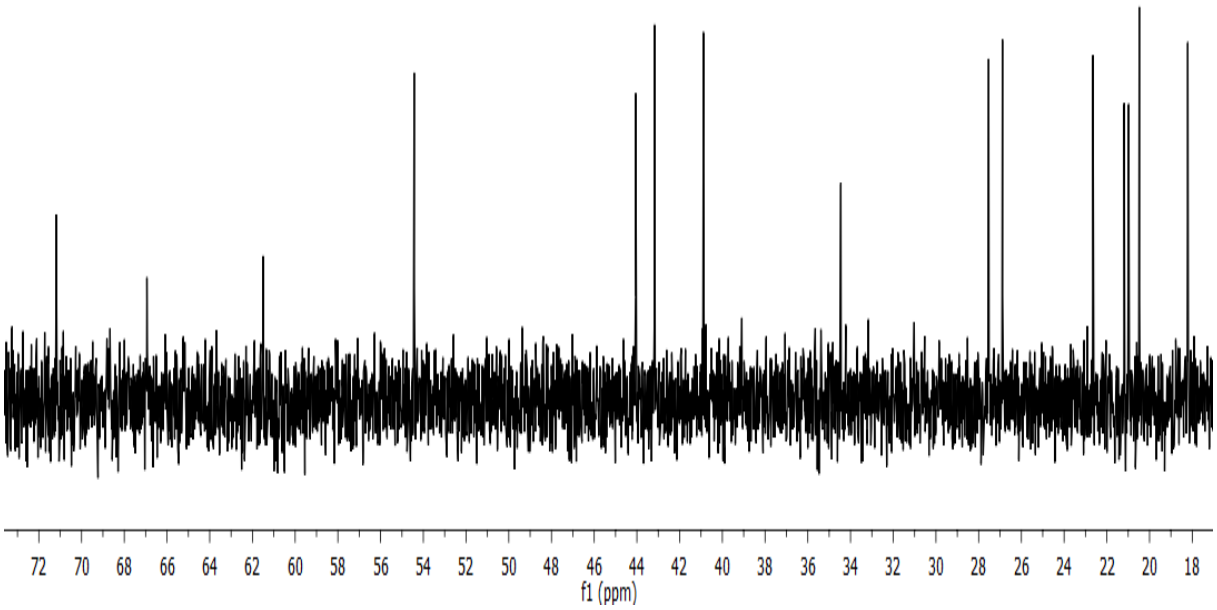

Figure S2. ^{13}C NMR spectrum of 7β,11β-epoxy-eudesman-4α-ol (**48)**

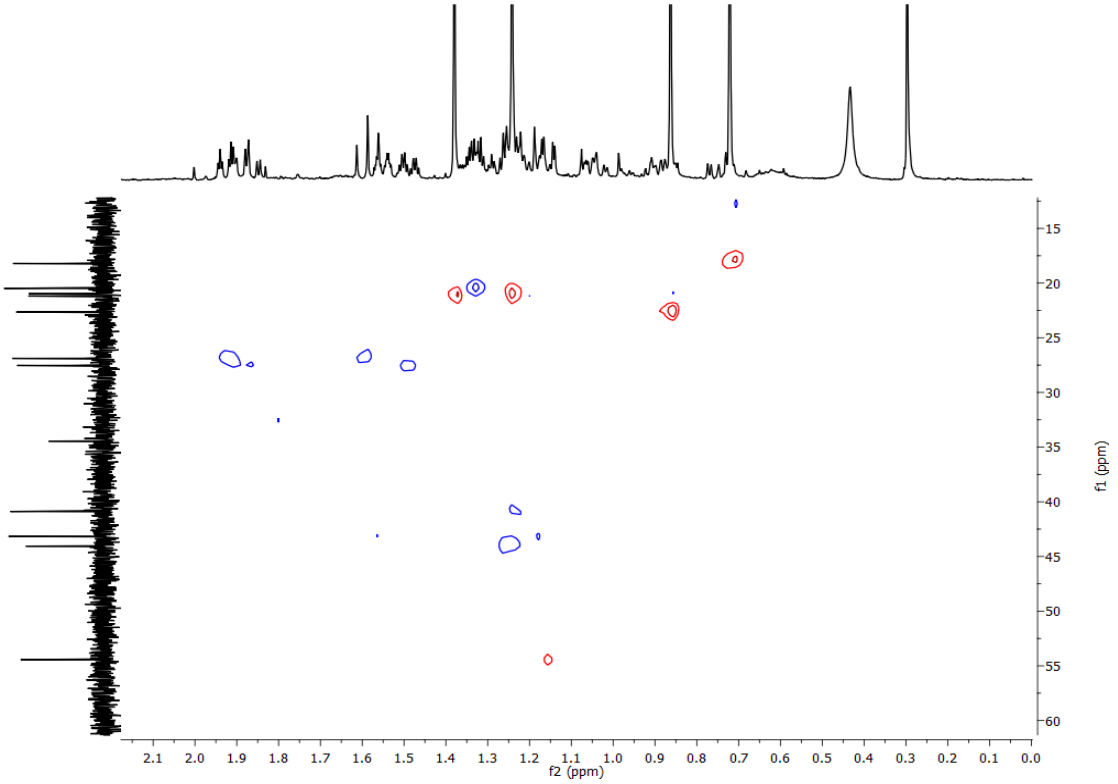

Figure S3. HSQC NMR spectrum of 7β,11β-epoxy-eudesman-4α-ol (**48)**

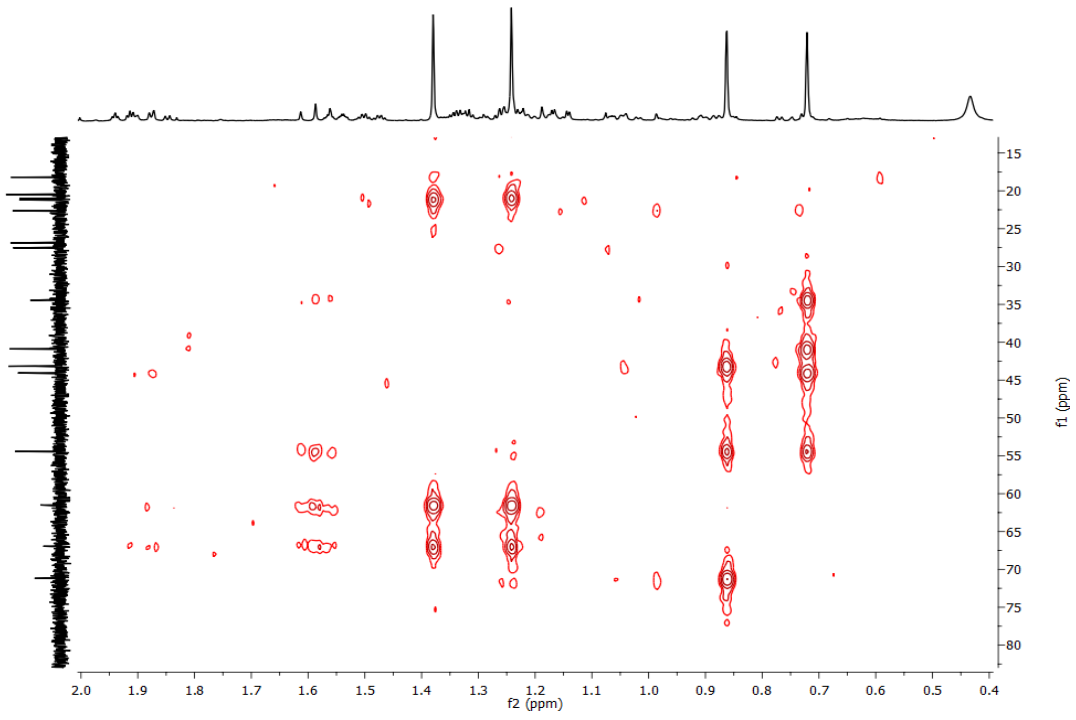

Figure S4. HMBC NMR spectrum of 7β,11β-epoxy-eudesman-4α-ol (**48)**

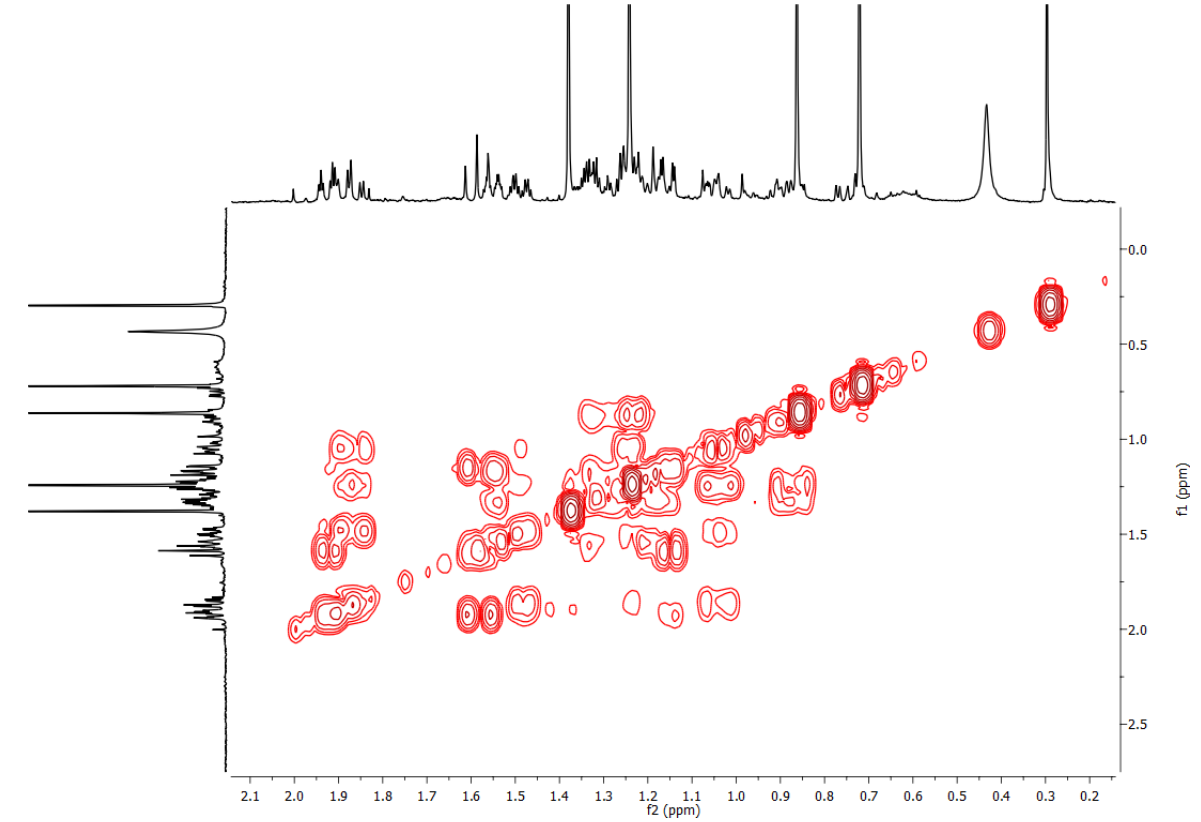

Figure S5. COSY NMR spectrum of 7β,11β-epoxy-eudesman-4α-ol (**48)**

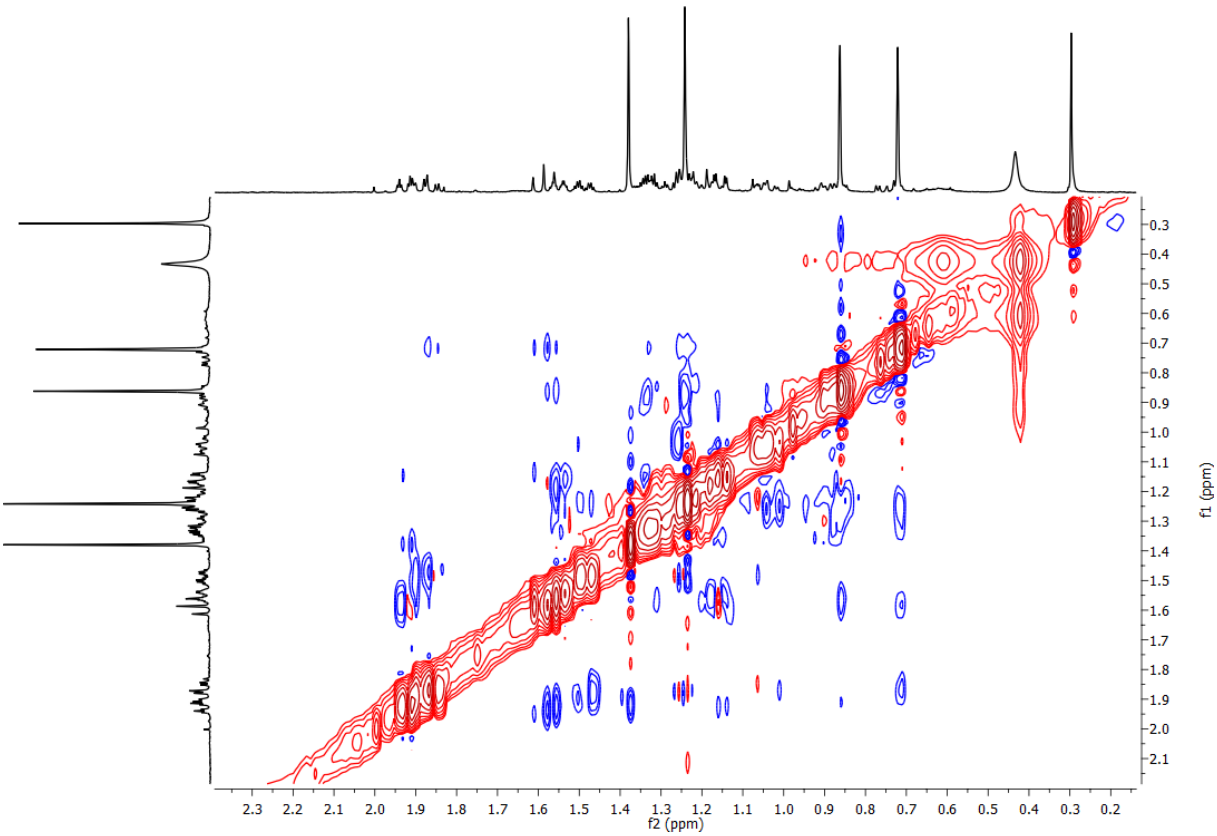

Figure S6. NOESY NMR spectrum of 7β,11β-epoxy-eudesman-4α-ol (**48)**

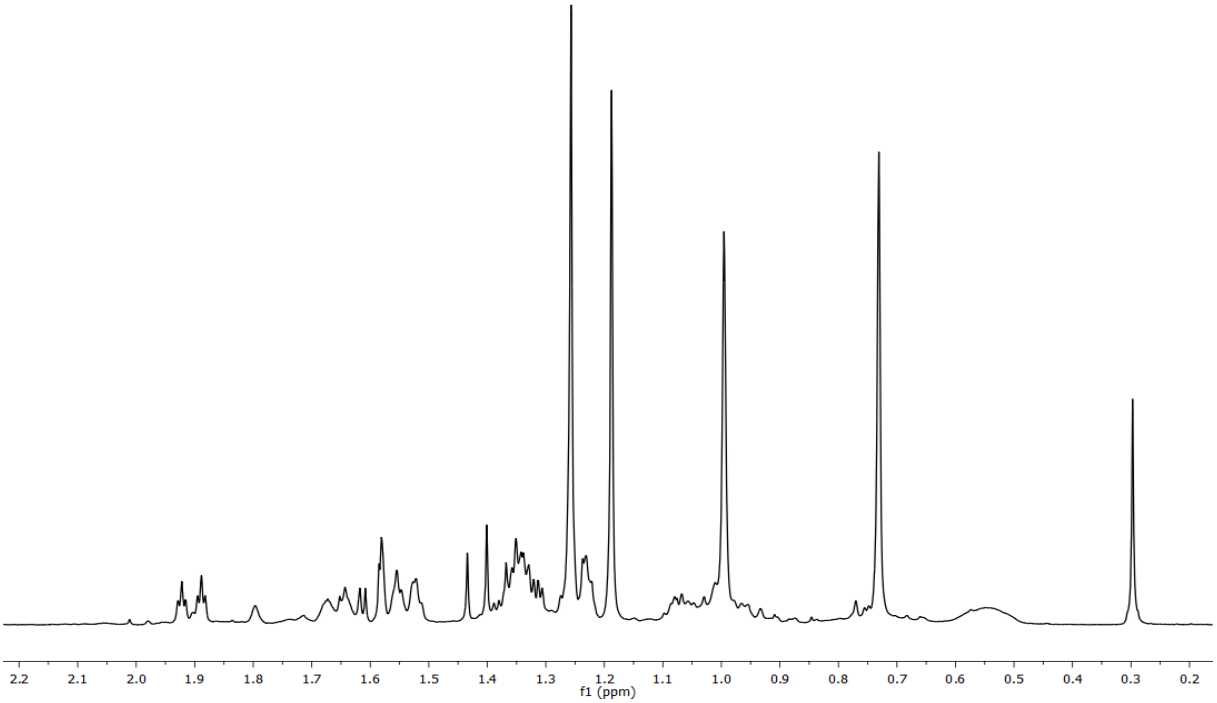

Figure S7. ^{1}H NMR spectrum of 7α,11α-epoxy-eudesman-4α-ol (**49)**

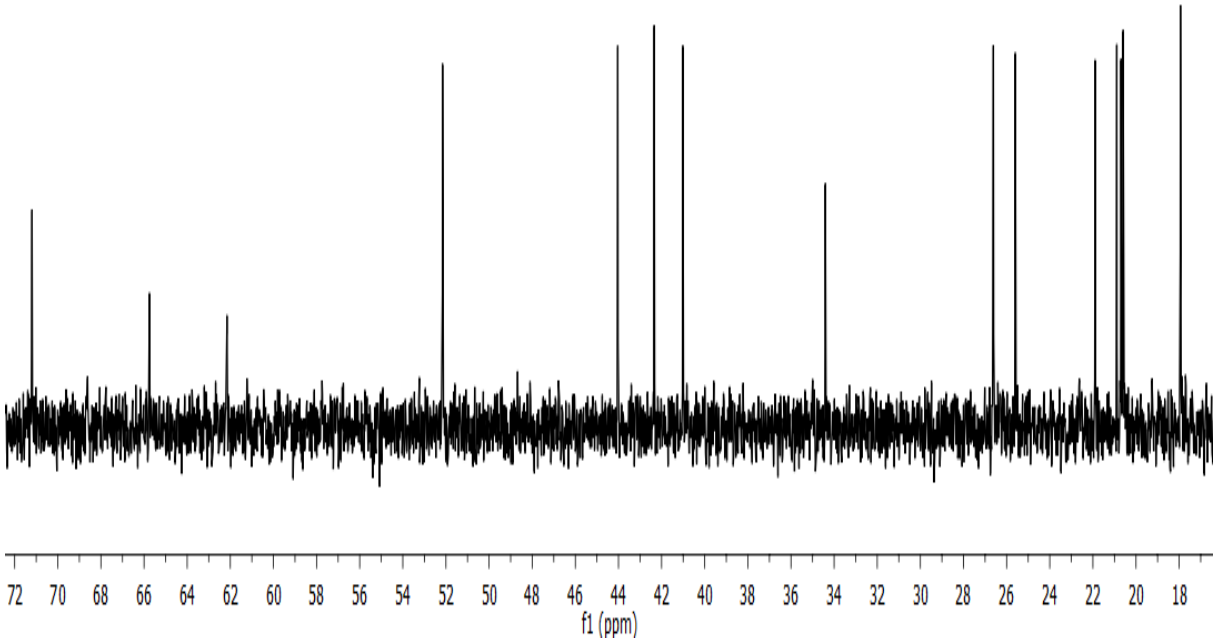

Figure S8. ^{13}C NMR spectrum of 7α,11α-epoxy-eudesman-4α-ol (**49)**

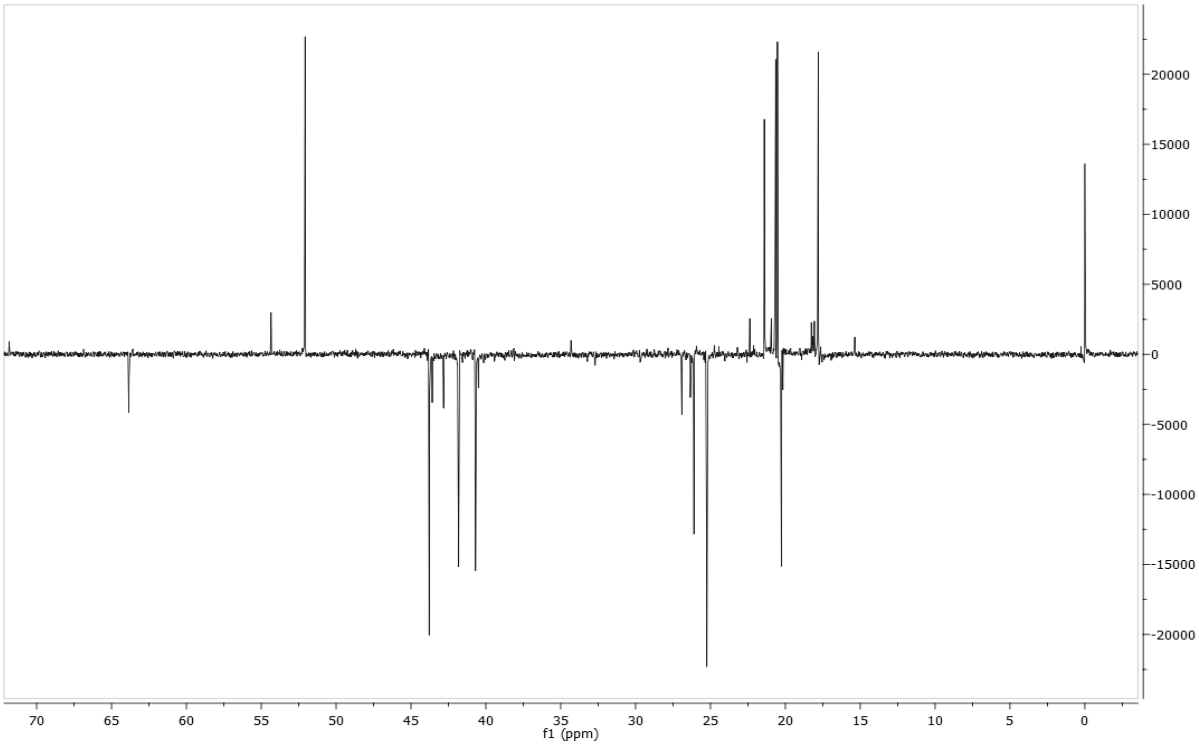

Figure S9. DEPT 135 NMR spectrum ($CDCl_3$) of 7α,11α-epoxy-eudesman-4α-ol (**49)**

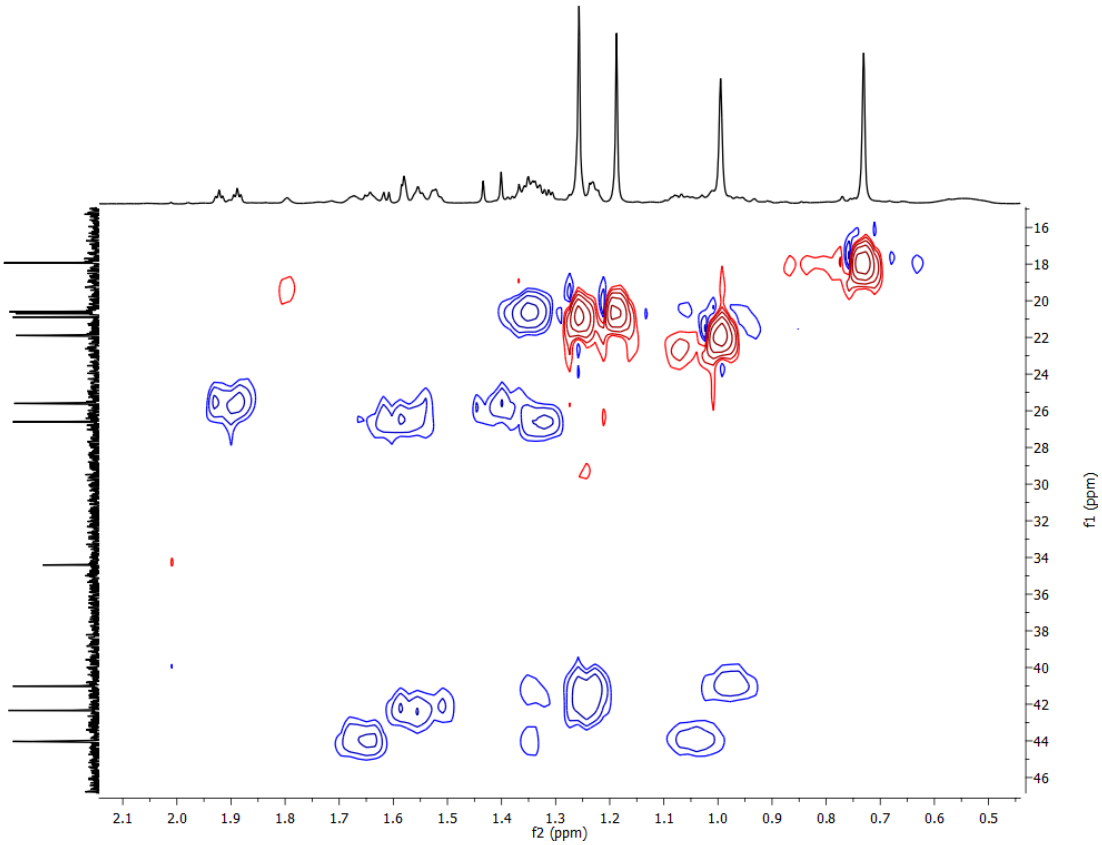

Figure S10. HSQC NMR spectrum of 7α,11α-epoxy-eudesman-4α-ol (**49)**

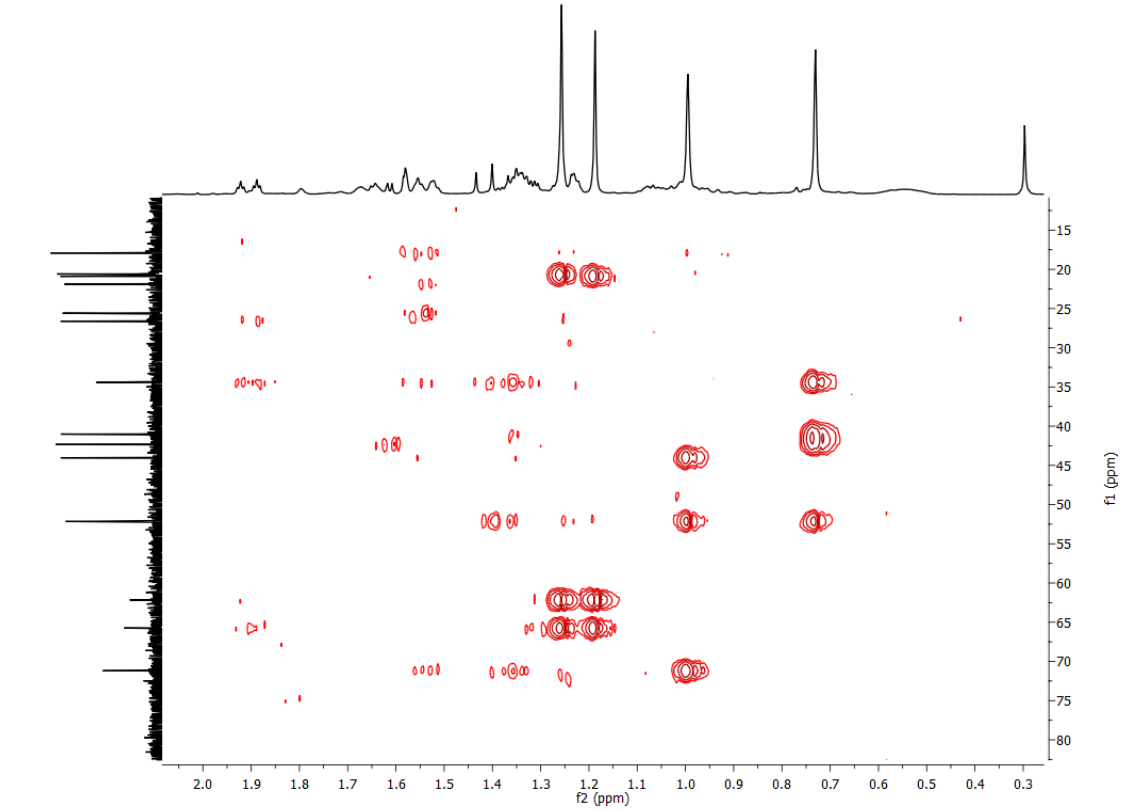

Figure S11. HMBC NMR spectrum of 7α,11α-epoxy-eudesman-4α-ol (**49)**

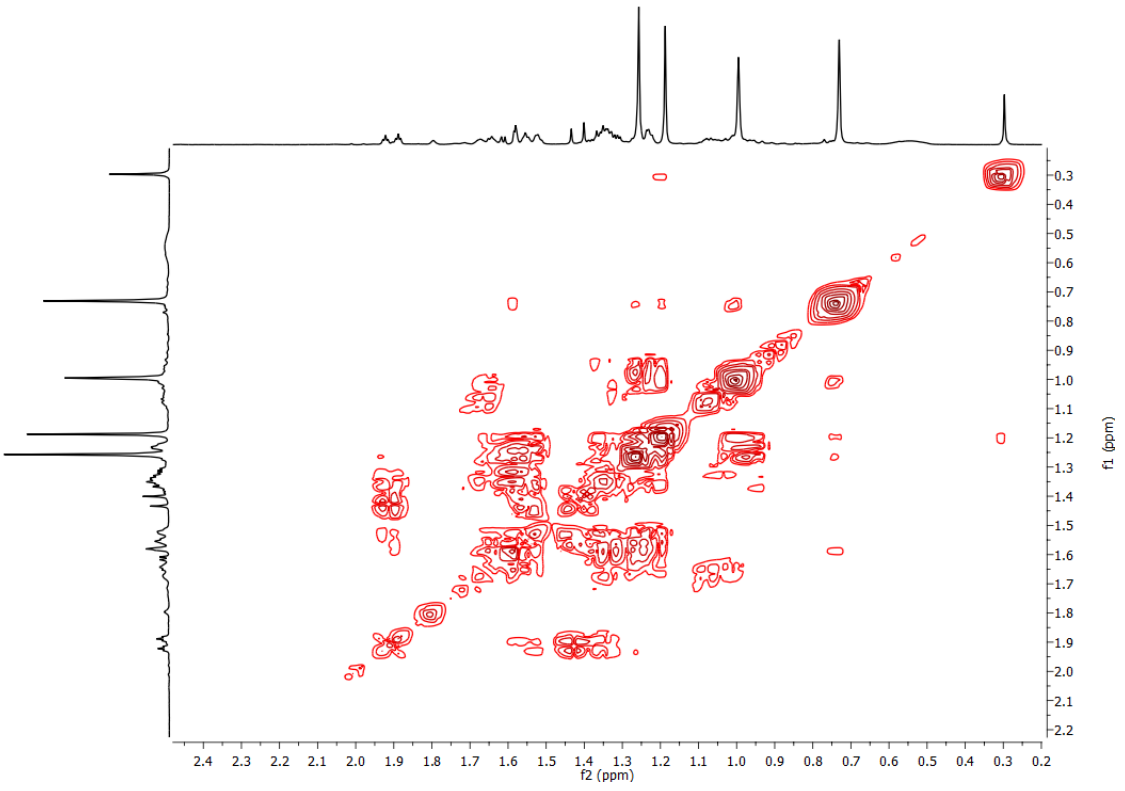

Figure S12. COSY NMR spectrum of 7α,11α-epoxy-eudesman-4α-ol (**49)**

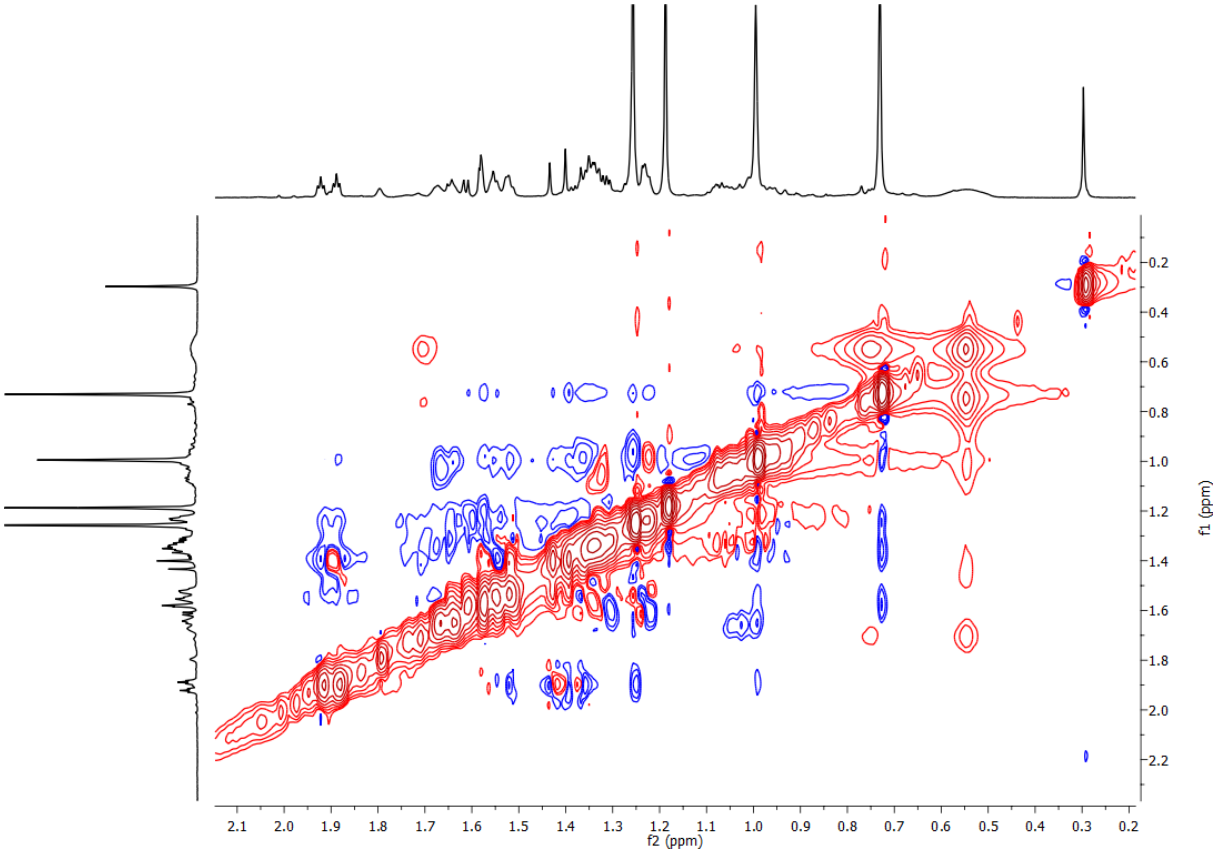

Figure S13. NOESY NMR spectrum of 7α,11α-epoxy-eudesman-4α-ol (**49)**

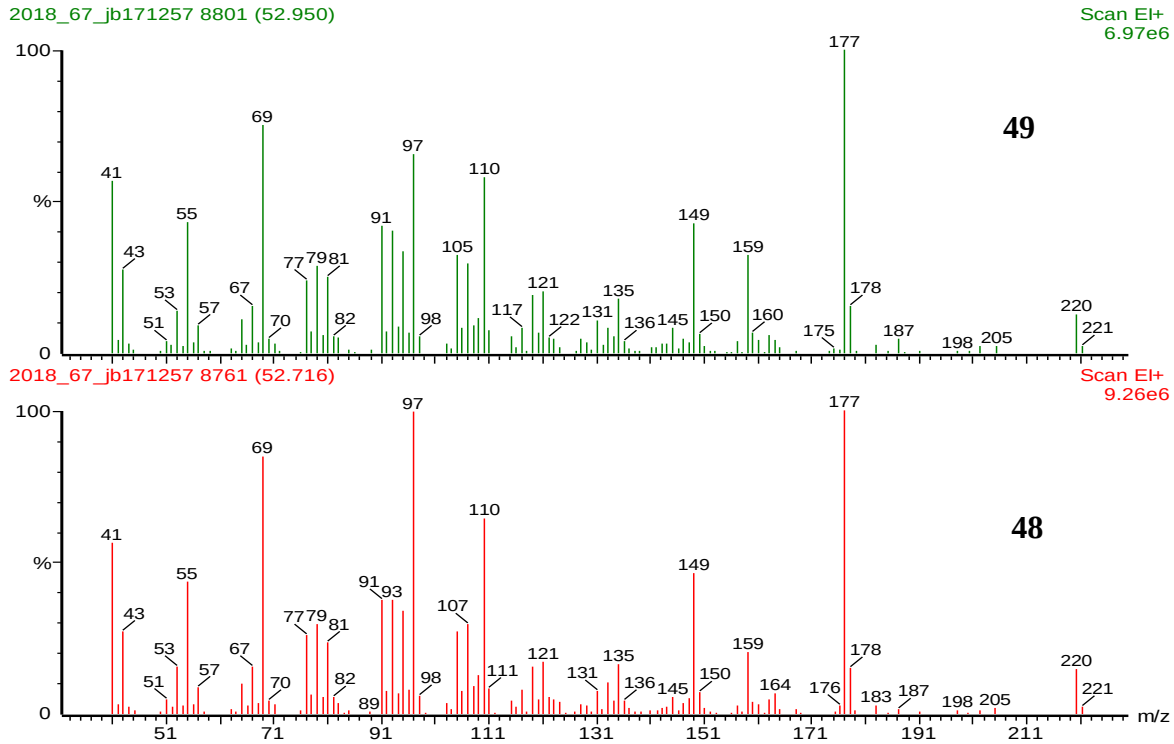

Figure S14. SM-IE spectra of 7β,11β-epoxy-eudesman-4α-ol (**48)** and 7α,11α-epoxy-eudesman-4α-ol (**49)**