
SUPPLEMENTARY INFORMATION

Usefulness of ^1H NMR multisuppression approach for the global characterization of monovarietal Extra-Virgin Olive Oils

Encarnacion Goicoechea-Oses, Ainhoa Ruiz-Aracama*

Food Technology, Faculty of Pharmacy, Lascaray Research Center, University of the
Basque Country (UPV/EHU). Paseo de la Universidad nº 7, 01006 Vitoria, Spain, Tel.:

34-945-013084. *E-mail: ainhoa.ruiz@ehu.eus

1. Quantification from ^1H NMR spectral data

The molar percentage of linolenic (C18:3 ω 3), linoleic (C18:2 ω 6), oleic (C18:1 ω 9) and saturated acyl groups (AG) in relation to the total number of moles of acyl groups present in olive oils were estimated as follows, from standard ^1H NMR spectra:

$$\text{Linolenic \%} = 100 * (A_{G2} / 3 * A_H) \quad [\text{eq.S1}]$$

$$\text{Linoleic \%} = 100 * (2 * A_{G1} / 3 * A_H) \quad [\text{eq.S2}]$$

$$\text{Oleic \%} = 100 * ((A_E - 2 * A_{G1} - A_{G2}) / 3 * A_H) \quad [\text{eq.S3}]$$

$$\text{Saturated \%} = 100 - \text{Linolenic\%} - \text{Linoleic\%} - \text{Oleic\%} \quad [\text{eq.S4}]$$

where A_E , A_{G1} , A_{G2} and A_H are the areas of signals **E**, **G1**, **G2** and **H** indicated in **Table 1**.

In the multisuppression approach, it must be noted that signal G due to bis-allylic protons was not suppressed, to be used for comparative purposes of the intensity of the signals between the standard and the multisuppressed spectra. Thus, the concentration of the several minor components, expressed as $\mu\text{mol/mol}$ of total acyl groups, was estimated from multisuppressed ^1H NMR spectra by using the following general equation:

$$\text{Component } (\mu\text{mol/mol of TG}) = 1000000 * [(A_X / n) / (A_H / 4)] \quad [\text{eq.S5}]$$

where TG means triglyceride, A_X is the area of the signal selected for the quantification of each component, shown in **Table 1**, and n is the number of protons that generate each signal.

Table S1. Quantitative data obtained by ¹H NMR on the main and minor components of monovarietal Arbequina (ABQ), Arroniz (ARZ), Cornicabra (COR), Hojiblanca (HOJ) and Picual (PIC) extra-virgin olive oils.

	Molar percentage (%)				μmol / mol of triglyceride					
	Linolenic	Linoleic	Oleic	Saturated	Cycloeucalenol	4,4-dimethylsterols	Esters of 4,4-dimethylsterols	Gramisterol + Obtusifoliosol	Δ7-sterols	Δ5-sterols
ABQ 1	0.6	8.8	74.7	15.9	4.3	132.3	32.5	146.2	77.3	952.6
ABQ 2	0.7	10.5	73.0	15.9	1.2	142.6	41.6	115.6	79.9	1104.8
ABQ 3	0.7	9.7	74.5	15.2	-	94.1	40.8	179.3	80.6	1078.4
ABQ 4	0.6	9.4	75.5	14.6	8.8	176.6	42.4	138.6	74.0	1085.9
ABQ 5	0.6	8.3	75.8	15.3	4.9	128.1	38.2	56.8	85.2	1124.5
ABQ 6	0.6	10.6	71.9	16.9	7.4	251.8	47.4	106.0	115.0	1252.5
ABQ 7	0.7	8.0	77.1	14.3	4.6	208.0	67.1	77.3	89.2	1267.2
ARZ 1	0.5	7.2	82.9	9.4	12.3	556.5	167.8	241.7	181.4	1521.1
ARZ 2	0.7	7.9	82.1	9.4	19.6	778.1	223.0	252.2	210.9	1595.4
ARZ 3	0.6	7.7	82.4	9.4	20.5	668.4	154.9	60.8	180.7	1439.2
ARZ 4	0.6	8.5	80.3	10.5	34.6	1002.9	311.0	188.7	228.5	1409.7
ARZ 5	0.5	8.3	80.4	10.8	25.3	962.2	293.0	206.2	216.9	1576.7
ARZ 6	0.7	6.9	81.8	10.6	-	582.3	210.0	82.9	144.6	1588.8
COR 1	0.6	3.4	83.7	12.3	32.8	389.6	153.7	321.3	179.2	1687.4
COR 2	0.5	2.8	85.6	11.1	12.6	183.2	47.9	243.6	105.7	1536.3
COR 3	0.6	3.7	83.7	12.0	24.2	423.5	132.6	279.2	180.3	1649.9
COR 4	0.6	2.8	85.7	10.9	26.4	183.0	55.1	257.9	125.3	1764.4
COR 5	0.6	3.5	84.7	11.3	34.0	322.0	137.3	240.3	147.1	1359.8
HOJ 1	0.9	6.0	81.3	11.8	5.6	150.3	72.6	222.0	148.2	1480.1
HOJ 2	0.7	5.5	79.6	14.3	20.9	362.2	95.7	400.1	196.5	1589.0
HOJ 3	0.7	5.1	82.8	11.5	20.6	374.7	133.0	296.2	141.9	1651.6
HOJ 4	0.8	5.8	82.4	11.0	3.3	179.1	98.6	271.0	167.0	1761.7
HOJ 5	0.7	7.0	79.0	13.2	33.8	297.5	132.7	211.9	134.1	1568.8
HOJ 6	0.8	6.1	83.2	10.0	20.7	190.1	94.4	372.9	187.0	1631.4
HOJ 7	0.8	5.7	81.6	12.0	7.0	110.7	44.1	363.9	164.9	1654.2
PIC 1	0.7	8.6	76.7	14.0	6.6	248.7	100.2	246.9	130.4	1491.7
PIC 2	0.7	4.3	82.4	12.6	25.8	465.7	152.9	238.6	120.0	1476.9
PIC 3	0.5	2.8	86.6	10.1	35.3	456.0	146.3	334.3	149.2	1312.6
PIC 4	0.6	3.6	85.2	10.7	45.1	361.0	125.6	337.6	137.1	1798.0
PIC 5	0.5	4.3	83.1	12.1	30.9	451.2	117.4	174.9	159.4	1323.5
PIC 6	0.5	3.0	85.9	10.6	0.1	305.0	60.3	372.3	124.1	1590.6
PIC 7	0.6	3.4	84.2	11.7	17.5	328.8	97.2	328.8	205.7	1565.8

Table S1. (continued)

	$\mu\text{mol} / \text{mol of triglyceride}$								
	Squalene	Non-cyclic diterpenic wax esters	Pinoresinol	1- Acetoxypinoresinol	5S,4S- oleuropeindial	Oleacein (3,4- DHPEA- EDA)	5S,4S- ligstrodiol	Oleocanthal (<i>p</i> -HPEA- EDA)	<i>E</i> -Elenolide
ABQ 1	6666.7	724.7	-	29.6	-	88.7	-	103.0	10.4
ABQ 2	7066.7	857.9	8.8	26.0	-	80.6	-	66.5	19.9
ABQ 3	6933.3	900.7	8.2	24.8	-	38.7	-	84.9	0.1
ABQ 4	6533.3	676.7	10.9	25.5	-	42.0	-	83.2	13.7
ABQ 5	8000.0	819.4	-	38.2	-	35.2	-	89.0	0.1
ABQ 6	7066.7	897.7	13.4	35.7	-	117.3	-	124.7	0.1
ABQ 7	9133.3	753.6	0.1	35.9	-	67.8	-	72.6	0.1
ARZ 1	19800.0	275.5	-	-	14.3	75.4	21.5	48.5	261.6
ARZ 2	20266.7	301.8	-	-	12.6	125.4	17.0	96.5	11.4
ARZ 3	20000.0	314.2	-	-	-	52.8	-	40.4	12.8
ARZ 4	17866.7	318.7	-	-	17.7	226.1	19.1	89.8	65.0
ARZ 5	18466.7	307.6	0.5	-	19.7	196.7	23.6	87.0	56.2
ARZ 6	16133.3	588.2	-	-	-	237.7	-	201.4	33.7
COR 1	17400.0	1187.9	17.9	-	-	15.1	20.4	78.1	-
COR 2	16333.3	847.0	22.7	-	-	160.0	61.0	257.7	41.3
COR 3	17200.0	871.6	13.2	-	-	109.3	12.7	197.6	13.4
COR 4	16800.0	833.4	13.5	10.9	-	17.4	7.2	195.8	62.3
COR 5	14066.7	826.1	11.9	10.2	-	98.0	10.9	230.8	0.1
HOJ 1	17200.0	240.9	-	11.9	-	-	-	-	-
HOJ 2	15533.3	411.2	-	-	17.4	157.6	31.2	253.1	49.7
HOJ 3	16066.7	430.6	-	12.3	-	126.7	-	132.5	53.5
HOJ 4	17800.0	301.0	-	13.8	-	-	-	-	-
HOJ 5	12066.7	729.1	13.4	30.8	-	83.7	-	113.1	13.6
HOJ 6	19466.7	164.8	-	29.3	-	110.5	26.5	165.2	39.8
HOJ 7	18666.7	309.2	-	22.6	-	58.7	23.5	121.5	111.2
PIC 1	11000.0	885.6	0.1	27.4	-	73.7	-	143.0	0.1
PIC 2	13933.3	290.7	6.6	-	-	68.2	12.0	105.8	0.1
PIC 3	15800.0	176.1	-	-	-	66.0	-	156.8	0.1
PIC 4	16800.0	613.5	19.8	-	-	-	-	-	0.1
PIC 5	17733.3	318.9	13.0	-	-	109.0	43.2	251.2	35.3
PIC 6	16333.3	146.5	0.1	0.1	-	101.3	-	193.0	0.2
PIC 7	17933.3	142.6	14.8	-	-	103.6	-	102.4	14.3

Table S1. (continued)

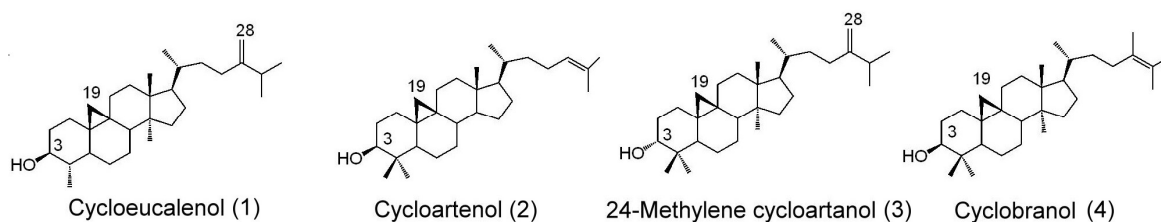
	$\mu\text{mol} / \text{mol of triglyceride}$							
	<i>p</i> -HPEA-EA	3,4-DHPEA-EA	Oleacein + Oleocanthal + Elenolic acid	Elenolic acid (estimated)	Oleokoronal	Oleomissional	Tyrosol (7.06 ppm)	Tyrosol derivatives (7.015 ppm)
ABQ 1	-	-	275.4	83.6	-	-	76.0	274.2
ABQ 2	-	-	151.9	4.9	-	-	36.3	173.1
ABQ 3	-	-	169.1	45.5	-	-	70.6	247.3
ABQ 4	-	-	150.1	24.9	-	-	60.2	223.5
ABQ 5	-	-	263.9	139.7	-	-	234.1	322.2
ABQ 6	-	11.3	242.1	0.1	-	-	58.1	325.2
ABQ 7	21.4	22.9	152.5	12.1	-	-	114.2	214.1
ARZ 1	8.3	55.1	274.9	151.0	25.2	75.3	134.2	311.4
ARZ 2	43.5	112.6	534.5	312.6	30.7	47.6	122.3	389.0
ARZ 3	21.2	120.7	347.7	254.4	29.7	41.1	120.5	240.9
ARZ 4	56.8	321.9	643.3	327.3	31.3	44.0	123.0	346.6
ARZ 5	41.3	278.1	646.1	362.4	37.9	68.4	200.8	416.6
ARZ 6	29.8	104.9	531.6	92.5	-	-	135.8	601.4
COR 1	39.9	50.9	292.6	199.4	68.3	-	706.1	541.0
COR 2	186.5	169.9	628.5	210.7	123.0	-	520.1	1339.6
COR 3	48.3	91.1	394.0	87.1	39.0	-	222.8	874.9
COR 4	31.4	29.4	340.2	127.0	33.6	-	200.8	645.9
COR 5	91.7	88.8	437.9	109.2	-	-	353.1	874.5
HOJ 1	48.3	-	197.4	197.4	14.3	-	221.6	-
HOJ 2	88.8	129.2	426.1	15.5	79.1	37.3	396.6	1288.6
HOJ 3	43.0	69.8	337.5	78.2	34.4	46.8	207.1	455.3
HOJ 4	-	39.0	164.7	164.7	21.1	-	321.9	214.4
HOJ 5	32.8	71.8	212.7	16.0	48.8	19.6	198.9	561.6
HOJ 6	18.8	47.1	427.4	151.7	18.1	-	236.5	648.0
HOJ 7	30.8	36.9	318.6	138.5	87.5	34.2	268.5	837.2
PIC 1	30.4	65.0	278.2	61.6	-	-	183.7	474.7
PIC 2	70.8	122.8	240.3	66.2	23.5	-	280.4	526.4
PIC 3	103.8	130.3	352.5	129.7	-	-	541.9	803.1
PIC 4	33.5	42.8	114.2	114.2	-	-	518.8	64.4
PIC 5	157.2	199.6	449.2	88.9	86.9	-	451.2	1227.2
PIC 6	96.5	178.1	415.7	121.4	116.1	-	194.9	877.7
PIC 7	68.3	136.3	251.2	45.2	61.4	-	373.1	642.0

Table S1. (continued)

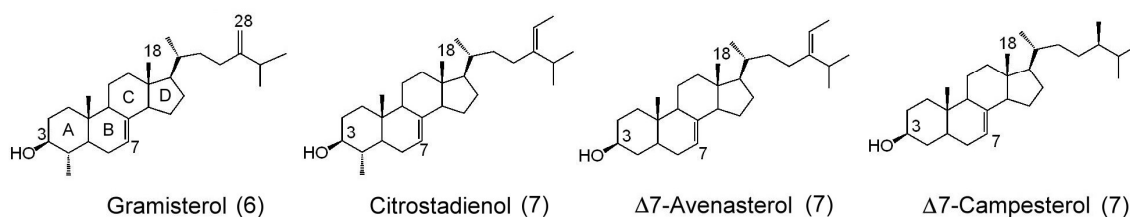
Estimated concentration (μmol /mol of triglyceride)				μmol / mol of triglyceride		
	Tyrosol derivatives (7.015 ppm)	Phenolics at 6.78 ppm: Tyr, Tyr derivatives, OHTyr and OHTyr derivatives	Phenolics at 6.60 ppm: Tyr derivatives, OHTyr and OHTyr derivatives	1,2-diglycerides	(<i>E</i>)-2-alkenals	Alkanals
ABQ 1	274.2	636.5	291.1	5806.8	24.2	48.4
ABQ 2	173.1	432.9	299.9	6609.0	40.9	63.7
ABQ 3	247.3	514.2	264.9	5054.9	28.5	66.4
ABQ 4	223.5	471.6	265.3	5923.7	26.4	66.1
ABQ 5	322.2	1018.1	333.8	5442.0	38.6	61.5
ABQ 6	325.2	684.0	552.2	5487.7	39.5	90.0
ABQ 7	214.1	612.3	314.1	5169.8	26.7	34.6
ARZ 1	311.4	1185.3	466.6	10348.4	17.1	51.6
ARZ 2	389.0	1270.2	492.8	9669.7	15.7	77.3
ARZ 3	240.9	1470.1	492.0	7890.2	12.0	61.2
ARZ 4	346.6	2148.3	1058.8	9706.9	12.8	74.5
ARZ 5	416.6	2110.8	1005.2	9938.8	7.2	75.5
ARZ 6	601.4	1479.9	900.3	6764.6	16.1	94.8
COR 1	541.0	1757.1	421.7	6331.1	22.0	46.2
COR 2	1339.6	2843.1	812.8	7610.3	26.6	40.7
COR 3	874.9	1716.9	736.4	6320.4	28.4	78.4
COR 4	645.9	1079.3	330.7	6145.3	10.3	48.0
COR 5	874.5	1887.6	638.3	4341.4	10.8	69.4
HOJ 1	-	1060.9	232.8	5406.4	18.8	37.1
HOJ 2	1288.6	2830.6	1083.4	8088.1	28.2	71.4
HOJ 3	455.3	1494.9	741.3	6646.7	9.4	72.1
HOJ 4	214.4	1128.5	290.3	6530.6	7.8	26.5
HOJ 5	561.6	1287.0	647.3	7152.2	23.9	78.8
HOJ 6	648.0	1382.4	587.2	8418.2	18.8	56.0
HOJ 7	837.2	1568.4	412.3	9262.6	10.9	76.0
PIC 1	474.7	1137.9	543.9	5883.2	18.3	128.3
PIC 2	526.4	1626.8	603.5	6377.3	11.6	91.4
PIC 3	803.1	2226.3	715.4	6062.6	-	86.0
PIC 4	64.4	1206.8	387.3	7418.1	13.9	97.3
PIC 5	1227.2	2718.1	805.9	7510.8	13.2	105.5
PIC 6	877.7	2045.2	646.4	8378.2	38.4	-
PIC 7	642.0	2111.0	813.8	7120.7	13.6	77.6

Sterols

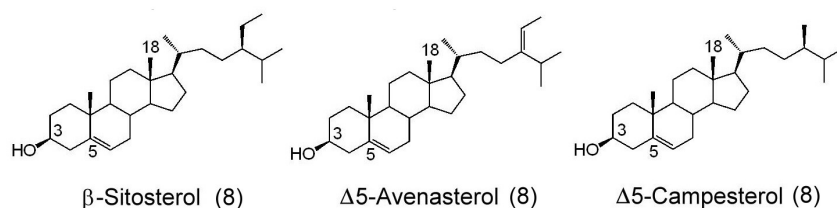
a) Sterols having a cyclopropane ring (protons in C-19)



b) Δ^7 -Sterols (double bond in C-7)



c) Δ^5 -Sterols (double bond in C-5)



d) Δ^8 -Sterols (double bond in C-8)

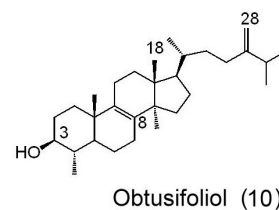
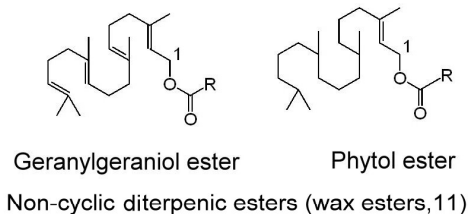
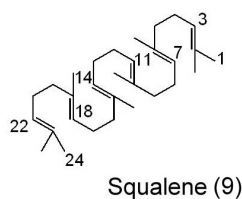


Figure S1. Chemical structures of olive oil sterols: **a)** Sterols having a cyclopropane ring (protons in C-19), such as cycloartenol, 24-methylenecycloartanol, cyclobranol and cycloeucalenol; **b)** Δ^7 -Sterols (double bond in C-7), such as gramisterol, citrostadienol, Δ^7 -avenasterol and Δ^7 -campesterol; **c)** Δ^5 -Sterols (double bond in C-5), such as β -sitosterol, Δ^5 -avenasterol and Δ^5 -campesterol; **d)** Δ^8 -Sterols (double bond in C-8), such as obtusifoliol. The carbon atoms are numbered in agreement with Table 1.

a) Terpenoids



b) Phenolic and secoiridoid derivatives

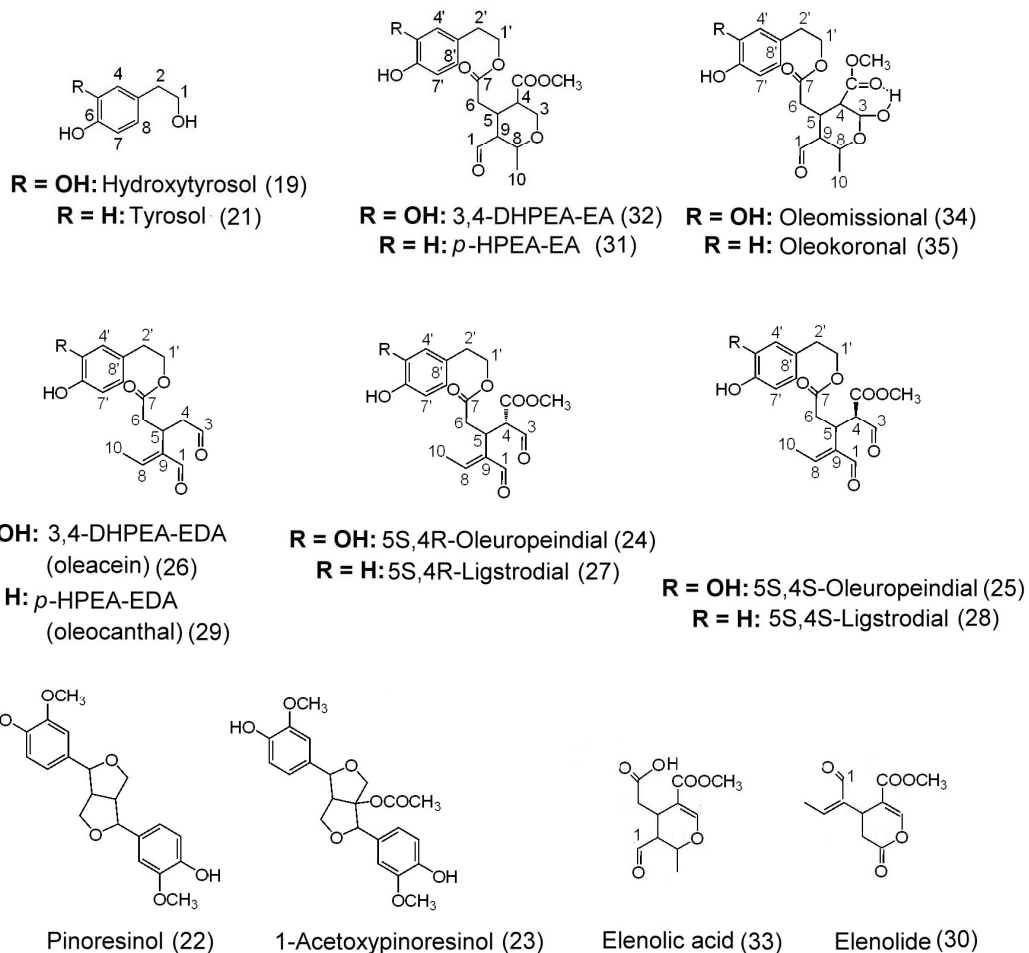


Figure S2. Chemical structures of **a)** Terpenoids, such as squalene and non-cyclic diterpenyl esters, like geranylgeraniol and phytol esters; **b)** Phenolic and secoiridoid derivatives. The carbon atoms are numbered in agreement with Table 1.

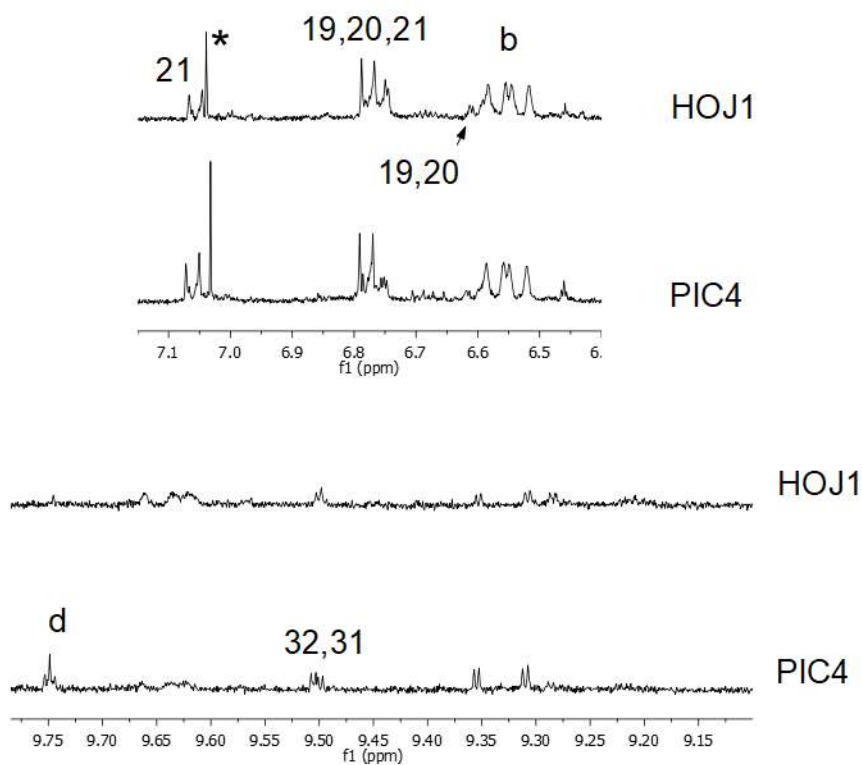
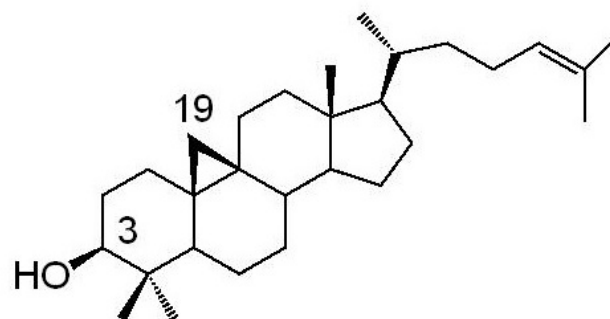


Figure S3. Two expanded regions of the ^1H NMR spectra of EVOO samples HOJ1 and PIC4, obtained using the multisuppression experiment (MS). Signal letters and numbers agree with those in Table 1. * Asterisked peak is a side band of chloroform.

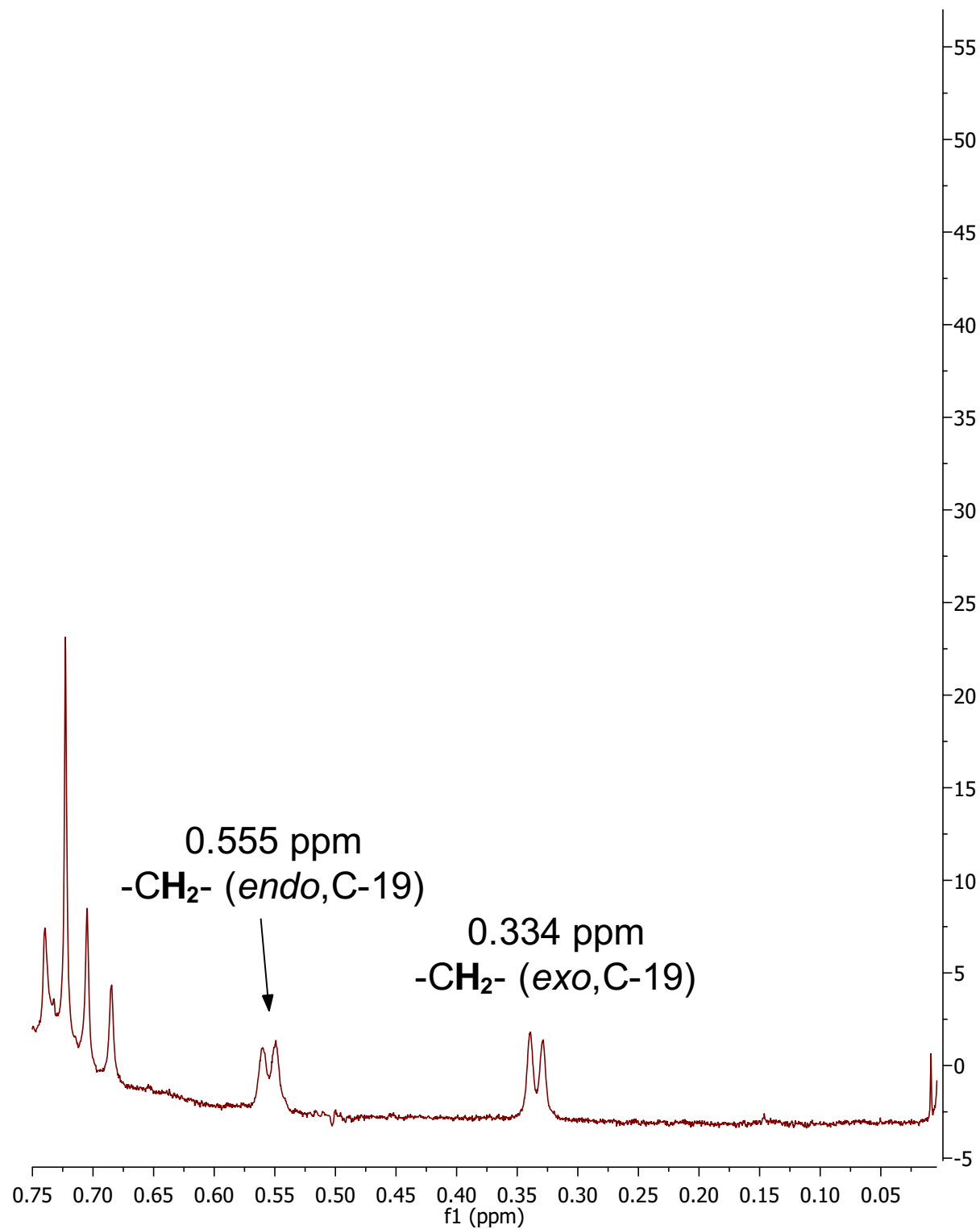
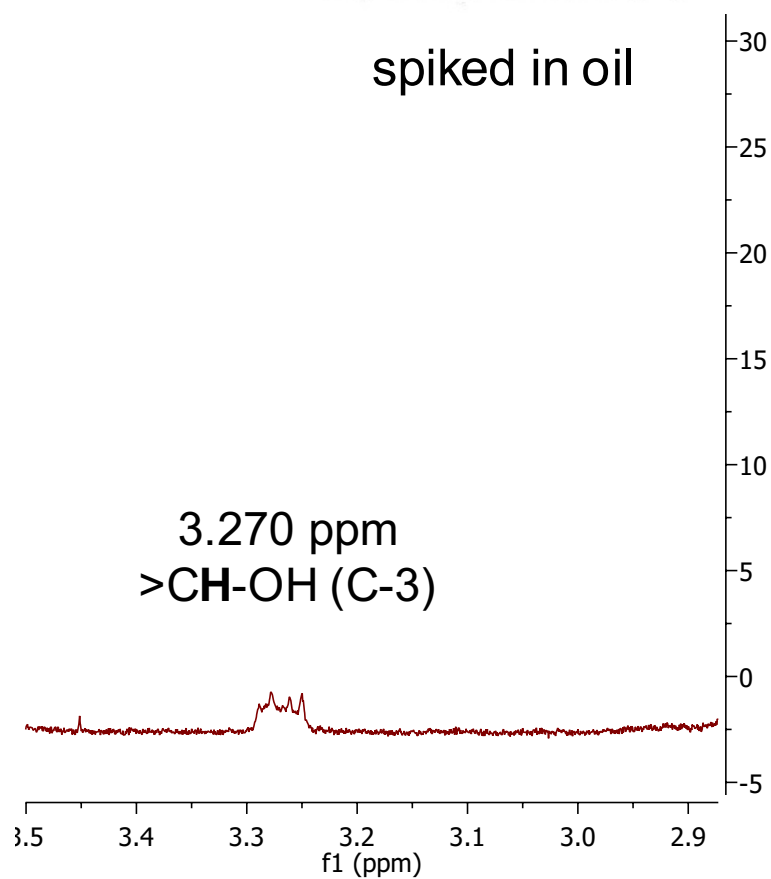
^1H NMR spectra of standards of some EVOO components spiked in oil (signal number or letter given in brackets):

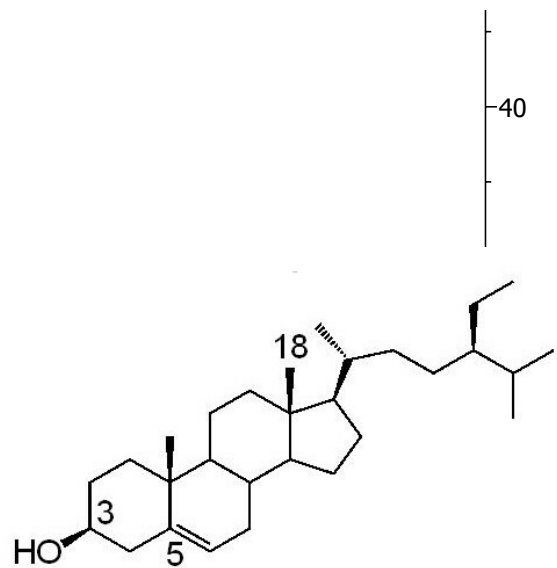
- Cycloartenol (2)
- β -sitosterol (8)
- Δ^5 -campesterol (8)
- Δ^5 -avenasterol (8)
- Squalene (9)
- Hydroxytyrosol (19)
- Tyrosol (21)
- Pinoresinol (22)
- 1,2-dioleoylglycerol (a)
- E-2-hexenal (c)
- Hexanal (d)



Cycloartenol (2)

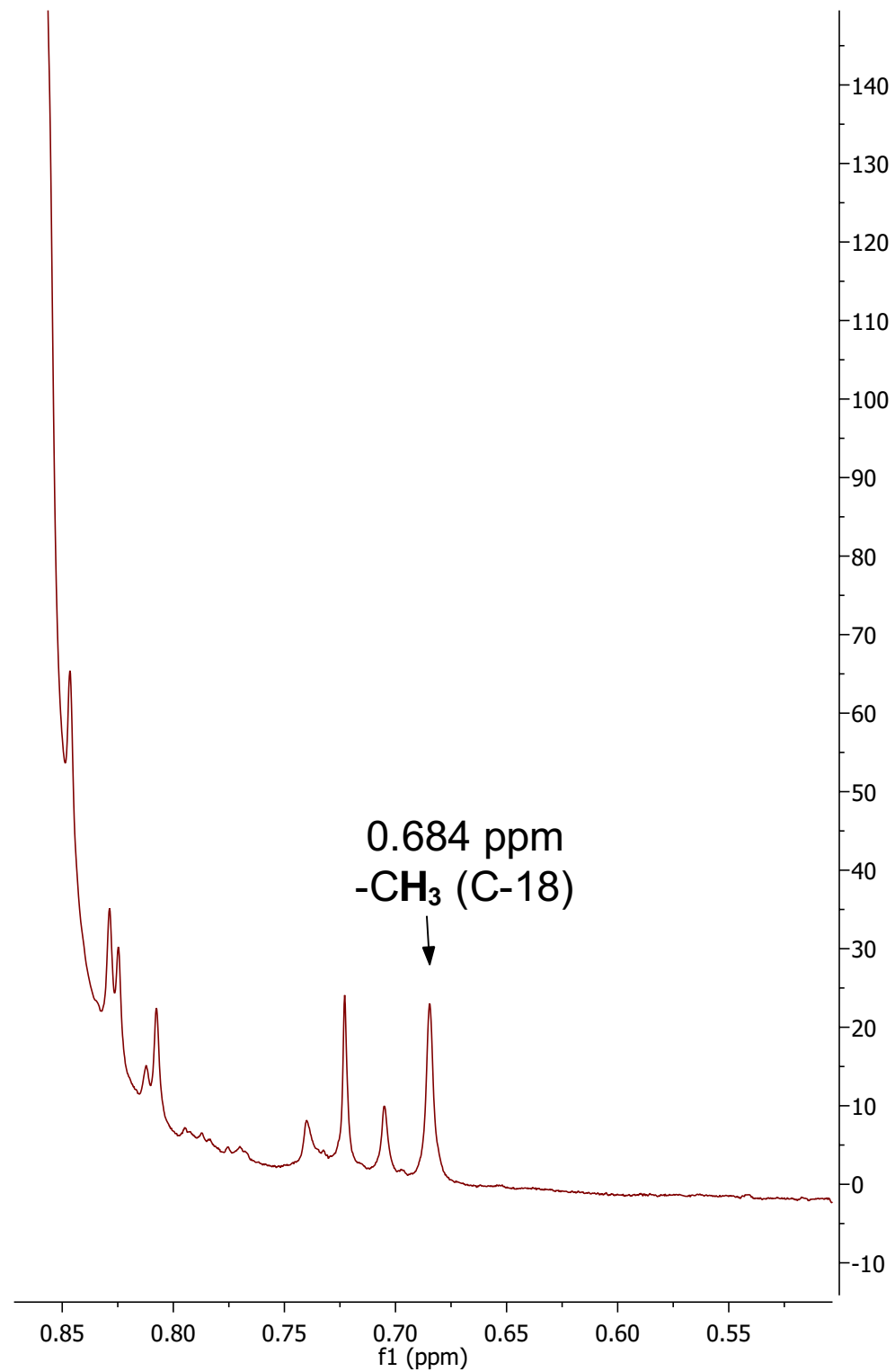
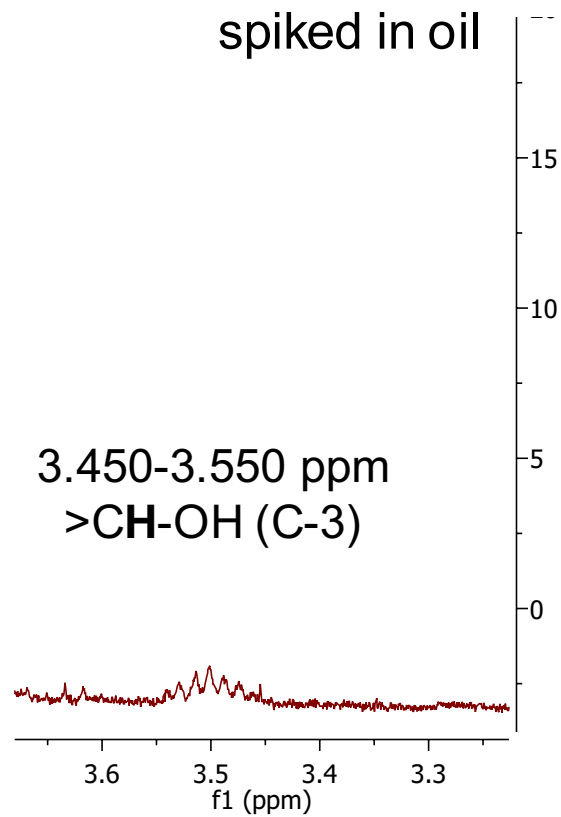
spiked in oil

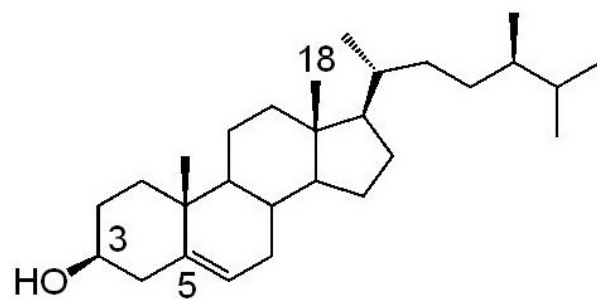




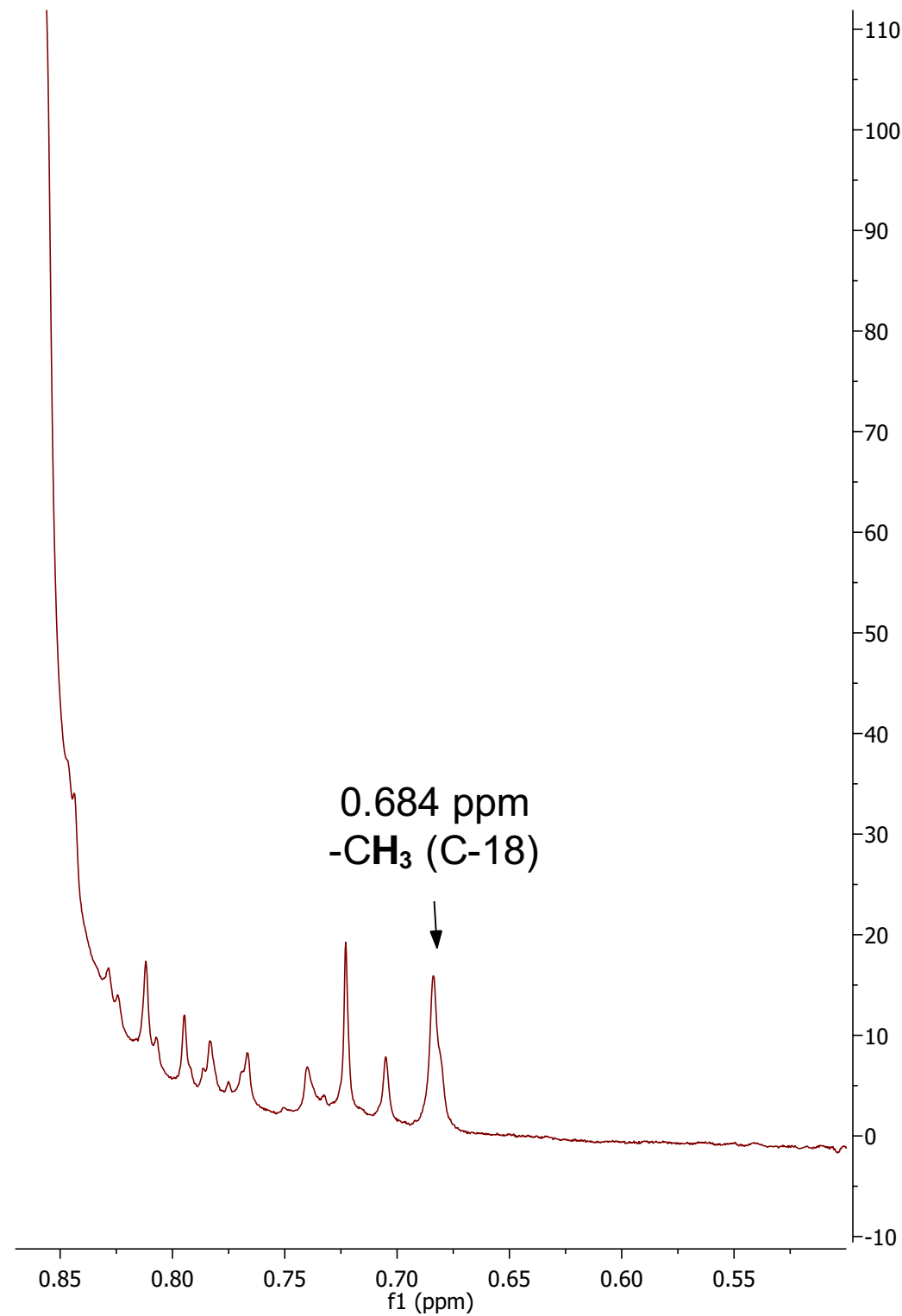
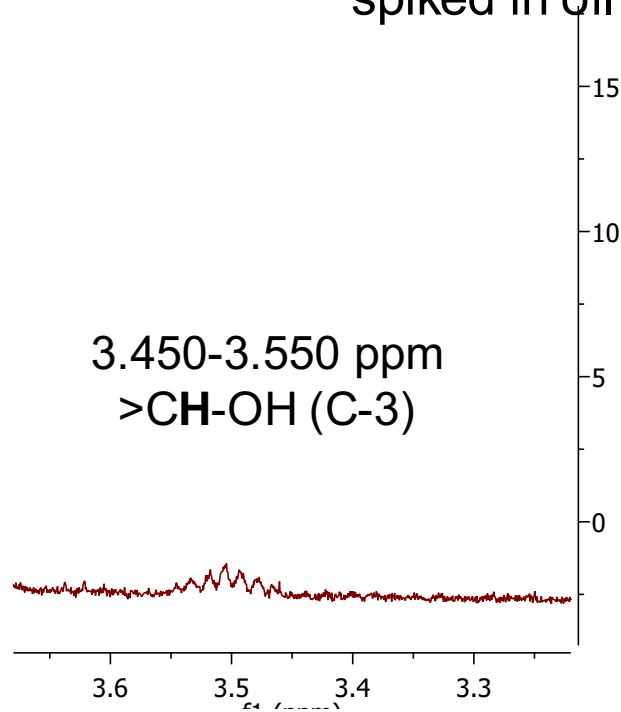
β -Sitosterol (8)

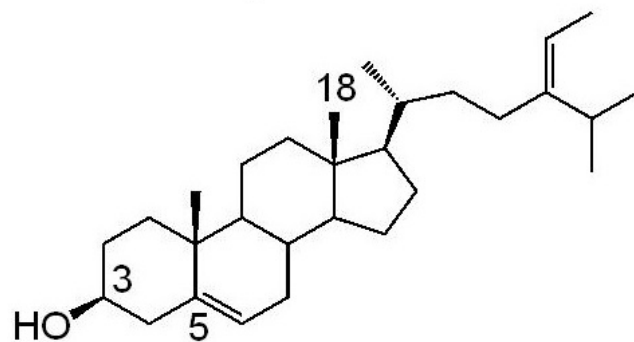
spiked in oil





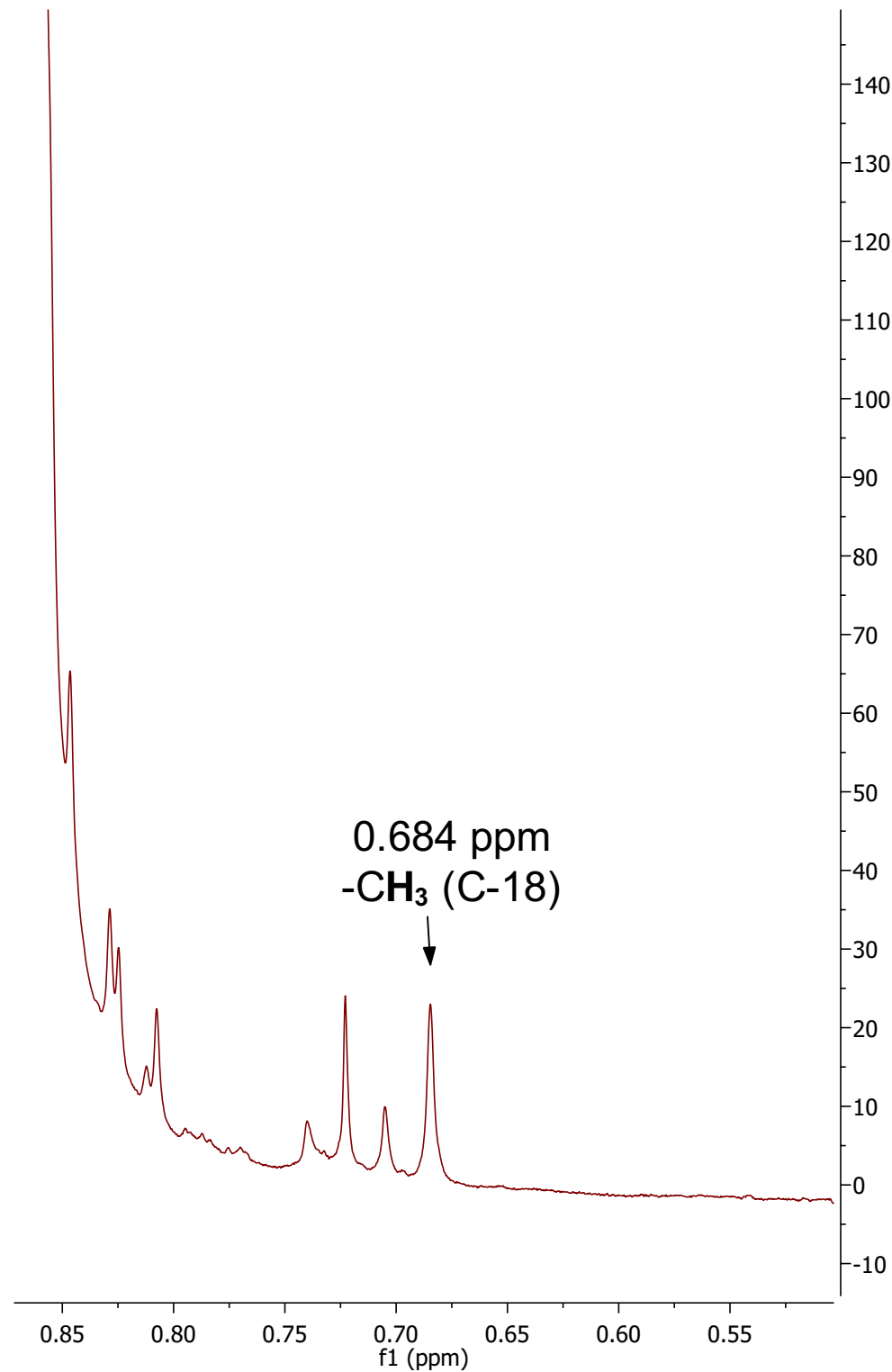
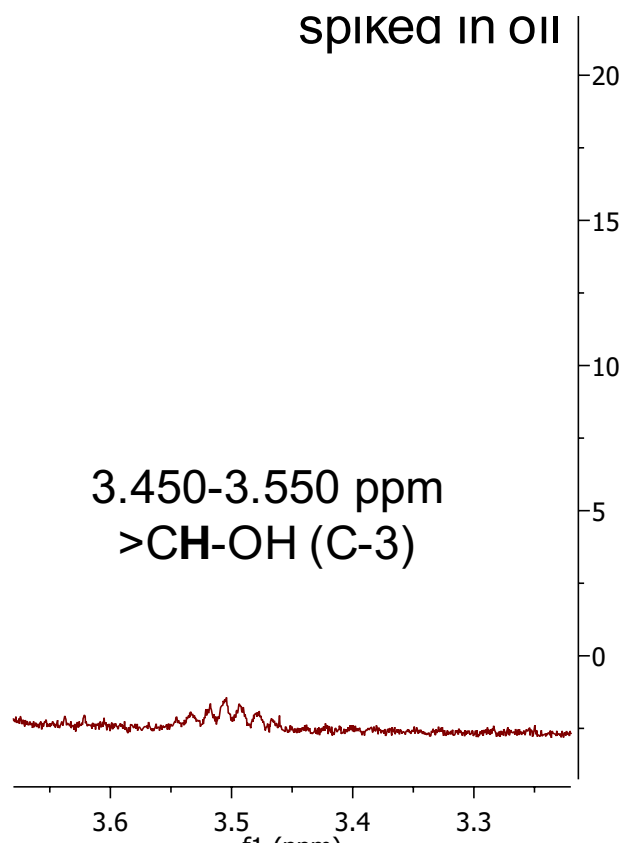
Δ^5 -Campesterol (8)
spiked in oil

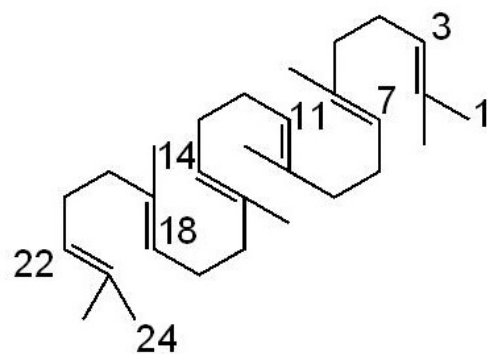




$\Delta 5$ -Avenasterol (8)

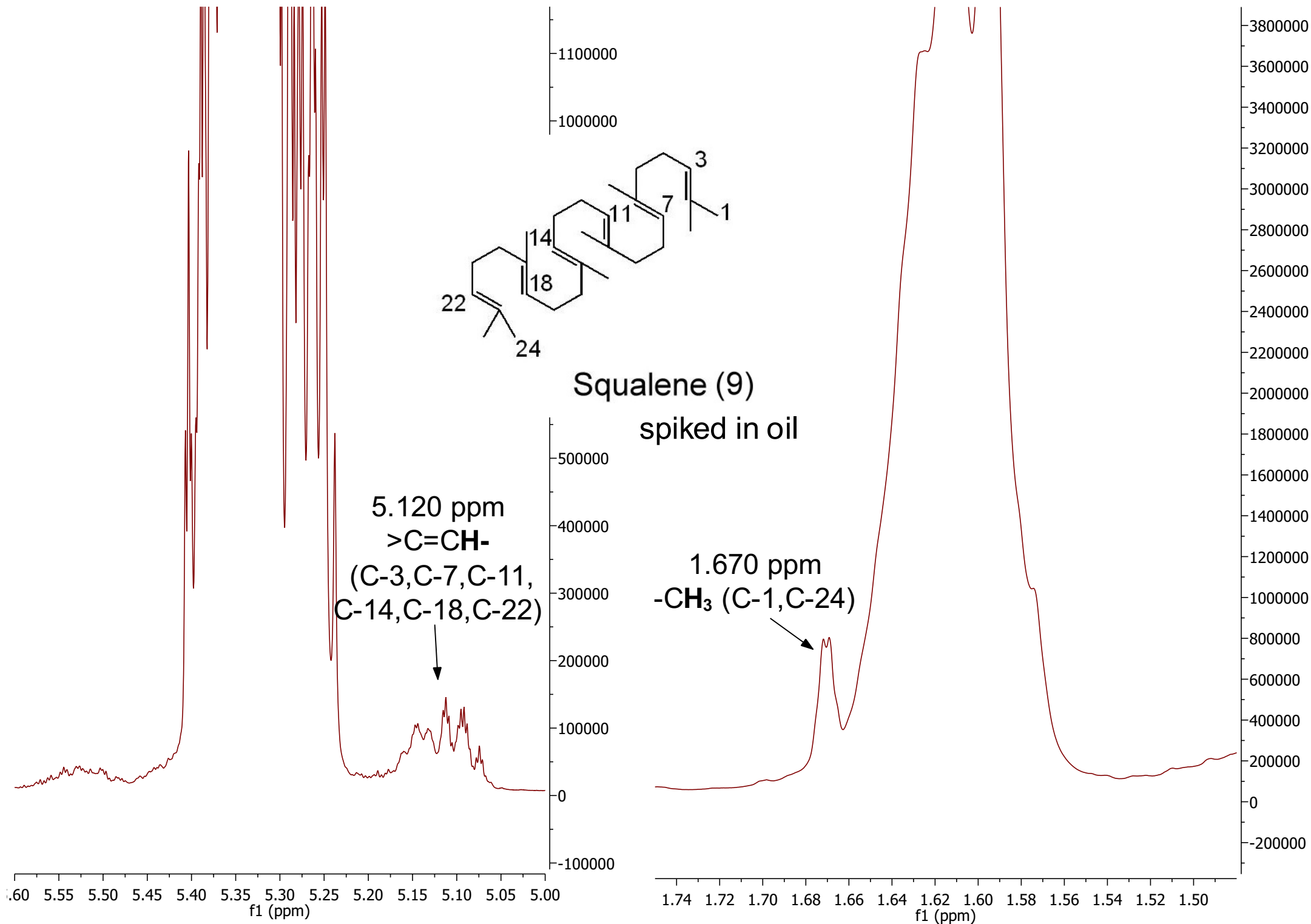
spiked in oil

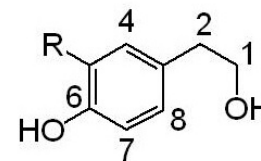




Squalene (9)

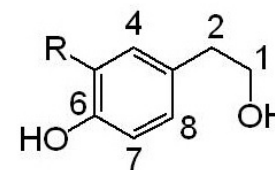
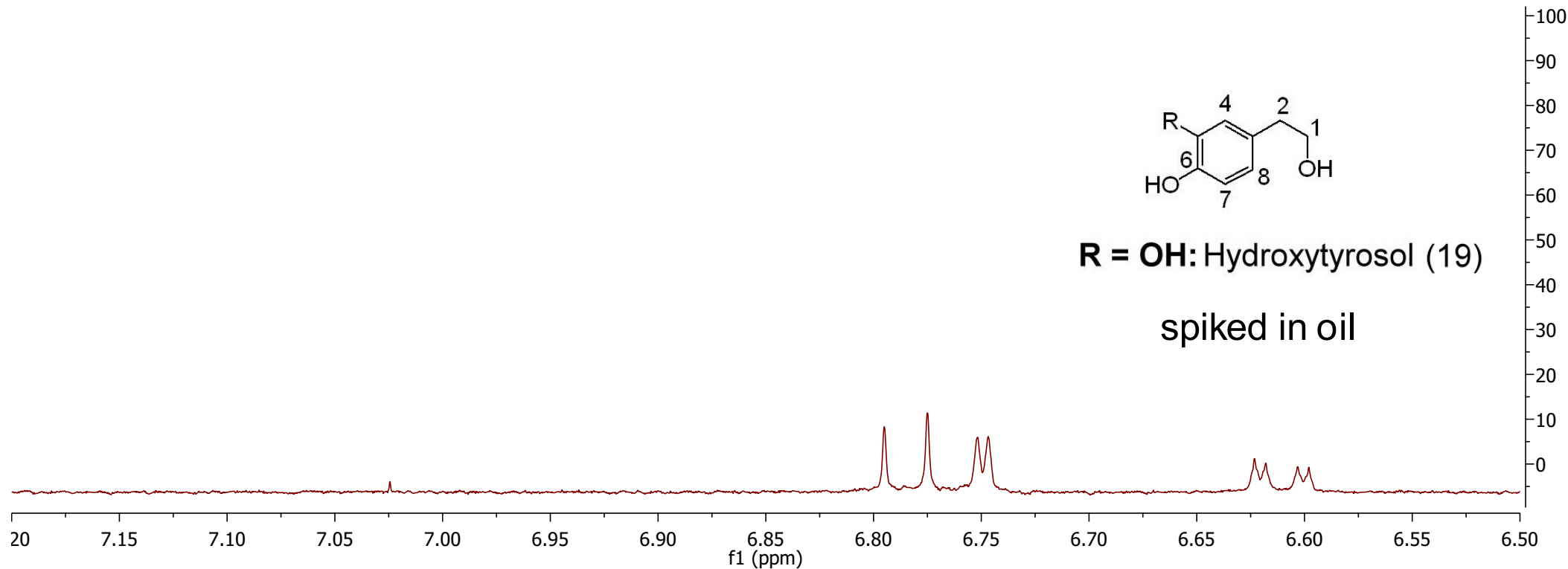
spiked in oil





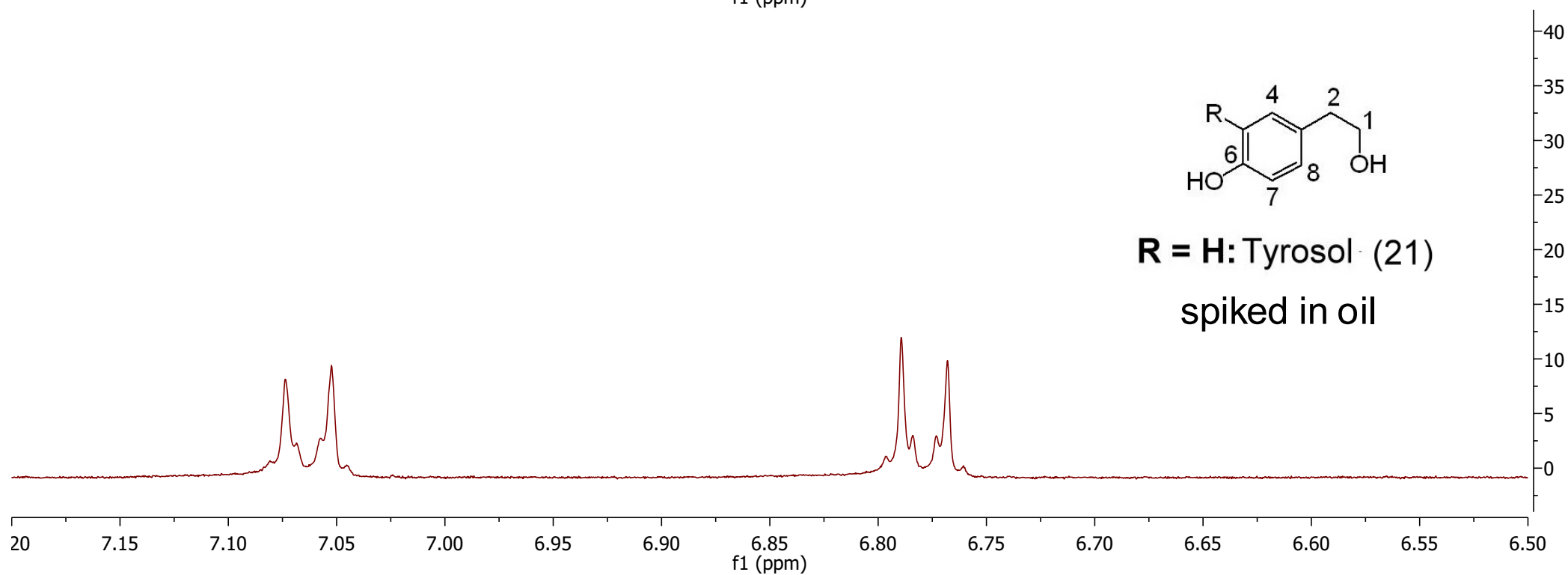
R = OH: Hydroxytyrosol (19)

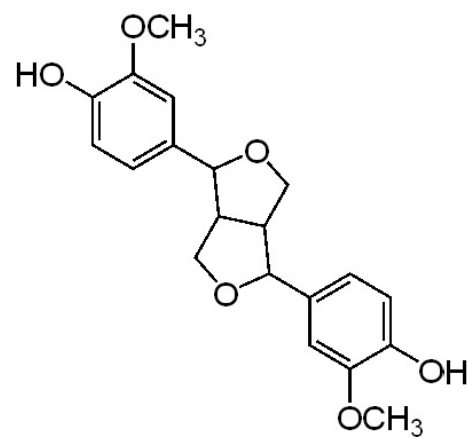
spiked in oil



R = H: Tyrosol (21)

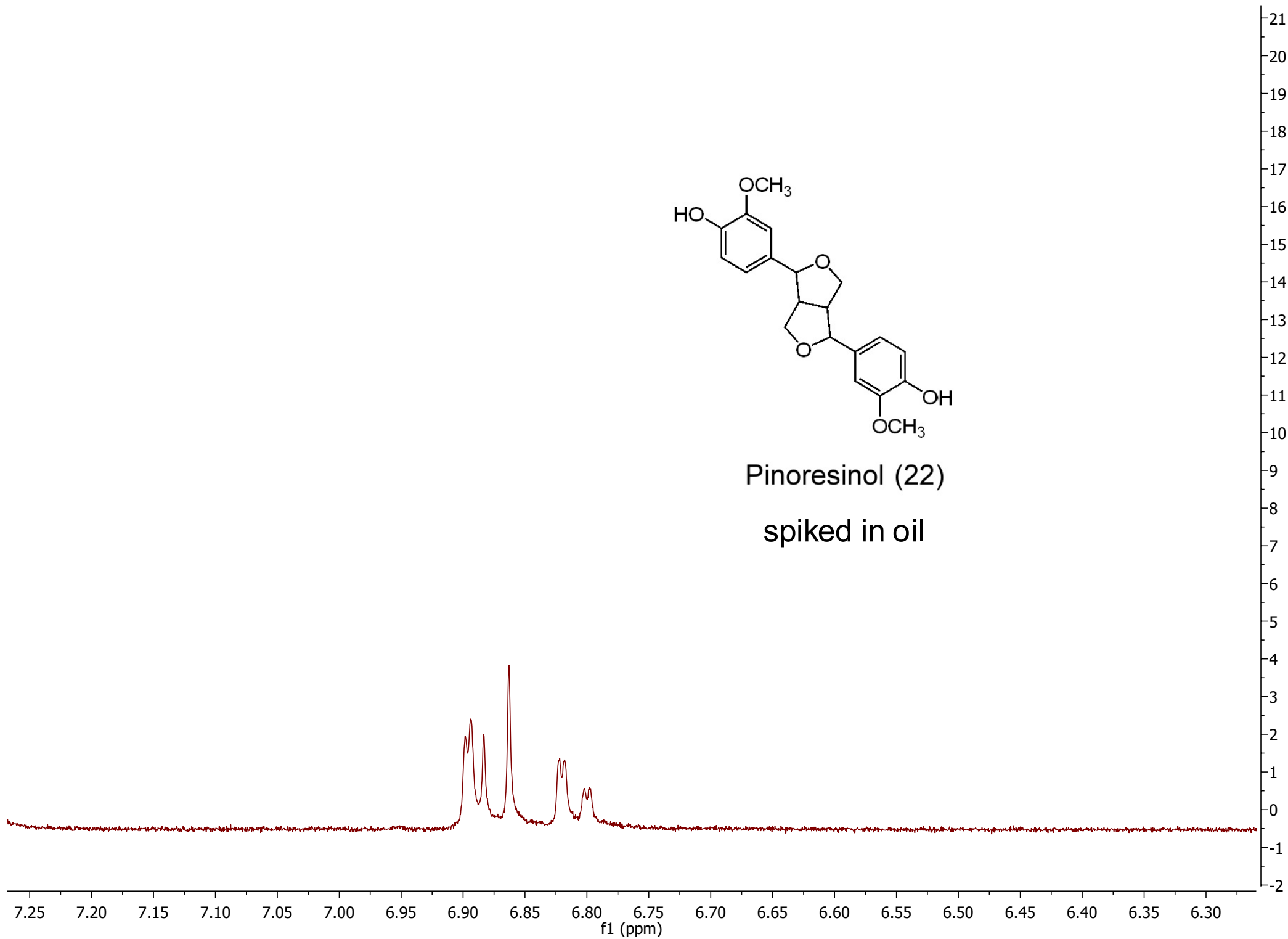
spiked in oil

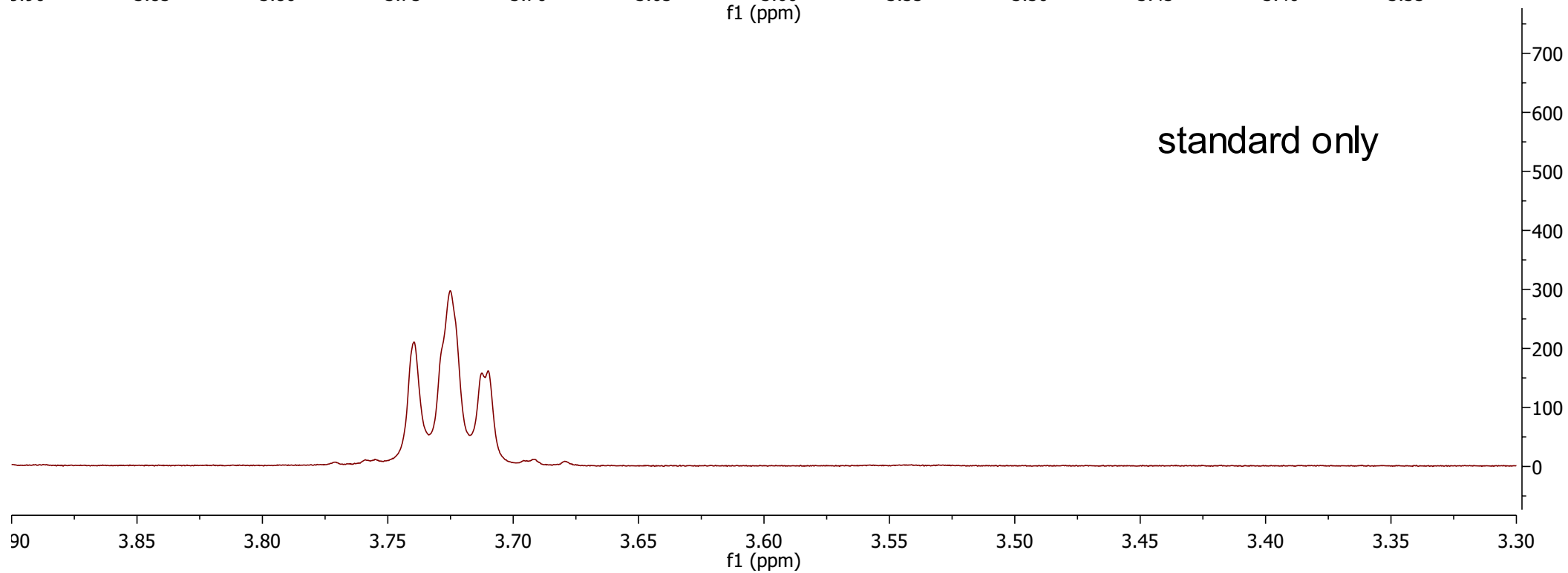
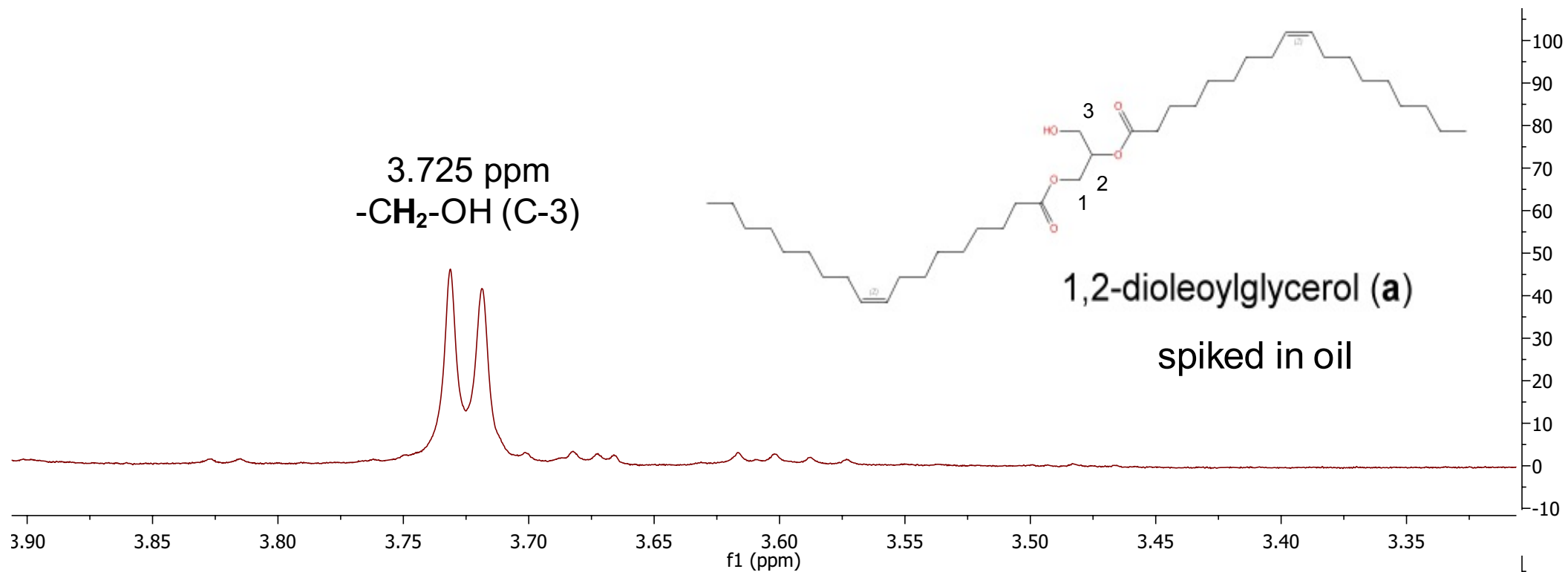


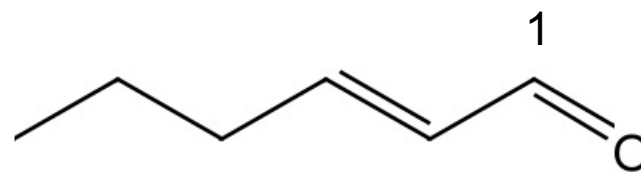


Pinoresinol (22)

spiked in oil



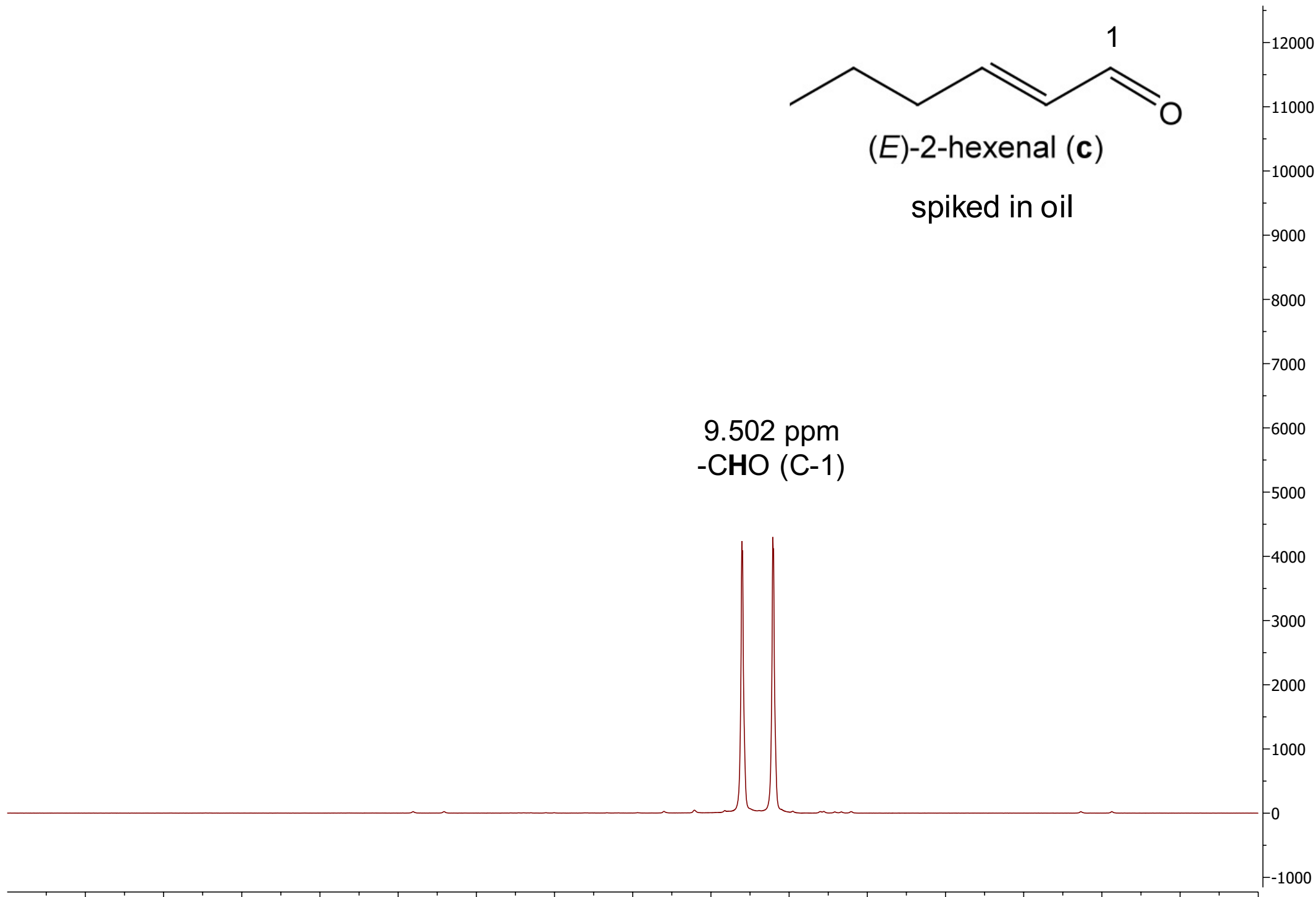


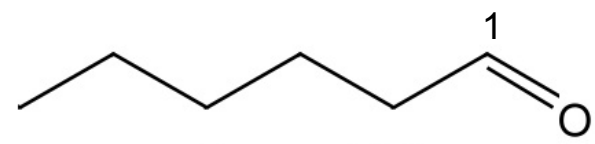


(E)-2-hexenal (c)

spiked in oil

9.502 ppm
-CHO (C-1)





Hexanal (d)
spiked in oil

9.750 ppm
-CHO (C-1)

