Supporting Information

***Rec. Nat. Prod*. X:X (201X) XX-XX**

Novel Hopanoic Acid and Depside from the Lichen *Dirinaria applanata*

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1. Supplemental data for structure elucidation of compound 1

Table S1. The comparison of NMR data of compound **1** with similar compound **1a**

Position	Compound 1		1β-Acetoxy-21α-hopane-3β,22-diol (1a) [1]	
	^{13}C-NMR (150 MHz)	^{1}H-NMR (600 MHz)	^{13}C-NMR (125 MHz)	^{1}H-NMR (500 MHz)
1	80.9	4.59 (1H, *dd*, 11.34 & 4.62 Hz)	80.9	4.59 (1H, *dd*, J = 11.5 & 5.0 Hz)
2	33.4	1.91 (1H, *m*, H_α)	33.4	1.62 (1H, *m*, H_a)
		1.62 (1H, *m*, H_β)		1.91 (1H, *m*, H_b)
3	75.2	3.31 (1H, *dd*, 12.24 & 4.26 Hz)	75.2	3.30 (1H, *dd*, J = 12.5 & 4.5 Hz)
4	38.8	-	38.8	-
5	53.0	0.65 (1H, *dd*, 11.58 & 2.04 Hz)	53.0	0.65 (1H, *dd*, J = 11.0 & 2.0 Hz)
6	17.9	1.57 (1H, *m*, H_α)	17.8	1.58 (*m*, H_a)
		1.49 (1H, *m*, H_β)		1.50 (*m*, H_b)
7	33.1	1.40 (1H, *m*, H_α)	33.0	1.42 (*m*, H_a)
		1.21 (1H, *m*, H_β)		1.22 (*m*, H_b)
8	42.2	-	42.2	-
9	50.7	1.45 (1H, *m*)	50.7	1.45 (*m*)
10	42.2	-	42.2	-
11	23.0	1.46 (2H, *m*)	23.0	1.46
12	23.8	1.46 (1H, *m*)	24.0	1.47 (*m*, H_a)
		1.32 (1H, *m*)		1.34 (*m*, H_b)
13	48.6	1.32 (1H, *m*)	49.3	1.34 (*m*)
14	41.9	-	41.9	-
15	33.5	1.16 (1H, *m*, H_α)	34.5	1.36 (*m*, H_a)
		1.32 (1H, *m*, H_β)		1.23 (*m*, H_b)
16	19.8	1.28 (1H, *m*, H_α)	21.9	1.93 (*m*, H_a)
		1.48 (1H, *m*, H_β)		1.57 (*m*, H_b)
17	53.6	1.25 (1H, *m*)	53.9	1.44 (*m*)
18	44.3		43.9	-
19	40.9	1.51 (1H, *m*, H_α)	41.2	1.50 (*m*, H_a)
		0.90 (1H, *m*, H_β)		0.91 (*m*, H_b)
20	26.6	1.43 (1H, *m*, H_α)	26.6	1.75 (*m*, H_a)
		1.87 (1H, *m*, H_β)		1.48 (*m*, H_b)
21	42.0	2.34 (1H, *m*)	51.1	2.20 (1H, *dt*, J = 11.0 & 8.5 Hz)
22	42.8	2.36 (1H, *m*)	73.9	-
23	27.9	0.97 (3H, *s*)	27.9	0.96 (3H, *s*)
24	15.0	0.77 (3H, *s*)	14.9	0.78 (3H, *s*)
25	12.8	0.98 (3H, *s*)	12.7	0.99 (3H, *s*)
26	16.9	0.94 (3H, *s*)	16.9	0.97 (3H, *s*)
27	16.6	0.91 (3H, *s*)	17.0	0.93 (3H, *s*)
28	15.7	0.70 (3H, *s*)	16.0	0.74 (3H, *s*)
29	183.6	-	28.7	1.17 (3H, *s*)
30	17.6	1.13 (3H, *d*, 6.48 Hz)	30.9	1.20 (3H, *s*)
1′	170.5	-	170.5	-
2′	21.9	1.99 (3H, *s*)	21.8	1.99 (3H, *s*)

**The highlighted rows showed the main differences between two compounds.*

2. Supplemental data for structure elucidation of compound 2

Compound **2** appeared as a white solid. The negative HRESI-MS gave a peak at 459.3840 $[M–H]^-$ (calcd. for $C_{30}H_{51}O_3^-$, 459.3843) which corresponded to chemical formula $C_{30}H_{52}O_3$. The FT-IR showed a hydroxy band at 3414 cm^{-1}. The ^{1}H-NMR indicated 8 singlet signals of methyl groups at δ_H 1.06 (3H, *s*, H-23), 1.16 (3H, *s*, H-24), 1.19 (3H, *s*, H-25), 1.30 (3H, *s*, H-26), 0.91 (3H, *s*, H-27), 0.77 (3H, *s*, H-28), 1.18 (3H, *s*, H-29), 1.21 (3H, *s*, H-30). Two peaks at δ_H 3.14 (1H, *m*, H-3) 3.14 (1H, *m*, H-6) belongs to two oxygen-bearing carbons which were also confirmed by the presence of two signals these at δ_C 79.1 (C-3) and 69.0 (C-6) in ^{13}C-NMR and DEPT. Further analyzing carbon spectra proved that there was an oxygenated quaternary carbon at δ_C 73.9 (C-22) characterized for a 2-hydroxy-2-propyl fragment. Based on these 1D-NMR characteristics, compound **2** can be reasonably inferred as a regioisomer of a hopanetriol [2].

To reveal the position of three hydroxy groups, HMBC analysis was recorded. The resulted spectra displayed that carbon at δ_C 79.1 was C-3 due to the correlation with neighboured protons namely H-1, 2, 5, 23, 24. Additionally, the cross-peaks between carbon at δ_C 69.0 and H-5, 7, 23 proved that this oxygenated carbon was C-6. Finally, the obtained data also supported for the presence of 2-hydroxy-2-propyl moiety at C-21 as indicated by the inter-correlations of H-17, 21, 29, 30 to hydroxyl carbon at δ_C 73.9 (C-22) [**Figure S1a**].

More specifically, the relative position of C-1, C-3 and C-21 were readily interpreted by analyzing NOESY spectrum. As shown in [**Figure S1b**], proton H-3 displayed nuclear overhauser effect with H-1α, 2α, 5α, 23 evidenced that the hydroxy group of C-3 must be *beta*- configuration. By using the same approach, carbon C-6 was similarly assigned for *beta*- configuration due to two important cross-signals from H-6 to H-5, 23. Carbon C-21 was clearly proved to be *alpha*- configuration because of the H-21/H-17 correlated signal. Based on the above evidence, compound **2** was solidly elucidated as 21α-hopane-3β,6β,22-triol.

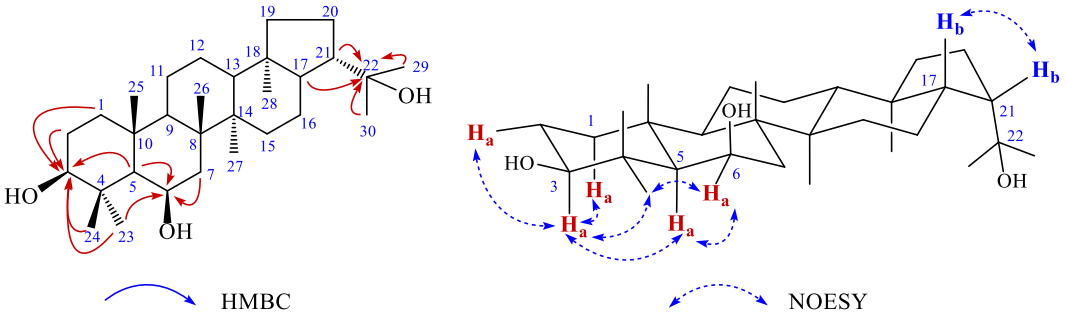
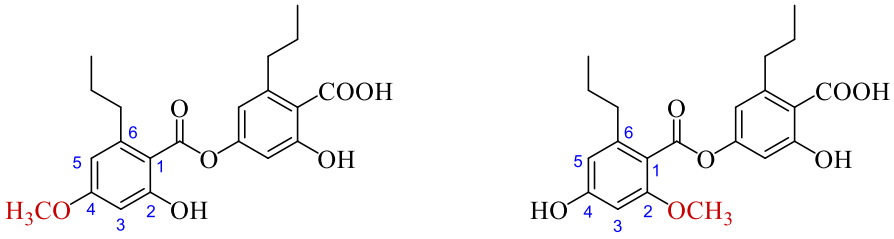


Figure S1: Some selected key HMBC (*a*) and NOESY (*b*) correlations of compound **2**

Table S2. The spectroscopic data of compound **2** ($CDCl_3$, δ in ppm, J in Hz)

No.	^{13}C-NMR (150 MHz)	^{1}H-NMR (600 MHz)	HMBC (^{1}H → ^{13}C)	NOESY (^{1}H → ^{1}H)
1	40.8	1.67 (1H, *m*, H$_\beta$)	C-2, 3, 5, 10, 25	H-1α, 25
		0.93 (1H, *m*, H$_\alpha$)	C-5	H-1β, 2β, 3, 5, 9
2	27.6	1.64 (1H, *m*, H$_\beta$)	C-1, 3, 4, 10	H-1α, 3, 24, 25
		1.57 (1H, *m*, H$_\alpha$)		H-3
3	79.1	3.14 (1H, *m*)	C-23	H-1α, 2β, 2α, 5, 23
4	39.6	-		
5	55.6	0.69 (1H, *m*)	C-3, 4, 6, 9, 10, 23, 24, 25	H-1α, 3, 6, 7α, 9, 23, 27
6	69.0	4.55 (1H, *s*)		H-5, 7α, 7β, 23, 24
7	41.0	1.72 (1H, *m*, H$_\alpha$)	C-5, 6, 8, 14, 26	H-5, 6, 7β, 27
		1.47 (1H, *m*, H$_\beta$)	C-5, 6, 9	H-6, 7α
8	42.0	-		
9	50.9	1.26 (1H, *m*)	C-12	H-1β, 5, 23
10	36.7	-		
11	24.2	1.45 (2H, *m*)	C-9, 13	
12	21.1	1.57 (1H, *m*, H$_\beta$)	C-13	
		1.46 (1H, *m*, H$_\alpha$)	C-9, 11, 13	H-27
13	48.8	1.47 (1H, *m*)	C-11, 12, 14	H-26
14	40.7	-		
15	34.5	1.44 (1H, *m*, H$_\beta$)	C-8	H-15α, 16β
		1.24 (1H, *m*, H$_\alpha$)	C-8, 12, 13, 17, 27	H-7β, 15β, 16β, 27
16	21.9	1.94 (1H, *m*, H$_\beta$)	C-8, 15, 17, 18	H-15β, 15α, 16α, 17, 30
		1.58 (1H, *m*, H$_\alpha$)		H-16β, 27
17	54.0	1.46 (1H, *m*)	C-16, 18, 22, 28	H-16β, 19β, 21
18	44.0	-		
19	41.3	1.55 (1H, *m*, H$_\alpha$)	C-17, 18, 20, 21, 28	H-19β, 28
		0.97 (1H, *m*, H$_\beta$)	C-13, 18, 20, 28	H-17, 19α, 20β
20	26.6	1.76 (1H, *m*, H$_\beta$)	C-17, 18, 19, 21	H-19β, 20α, 21
		1.50 (1H, *m*, H$_\alpha$)		H-20β, 28
21	51.1	2.23 (1H, *dt*, 10.8 & 9.0 Hz)	C-18, 22	H-17, 20β, 29, 30
22	73.9	-		
23	27.6	1.06 (3H, *s*)	C-3, 4,5, 6	H-3, 5, 6, 9, 24
24	16.9	1.16 (3H, *s*)	C-3, 4, 5, 23	H-2β, 6, 23, 25
25	17.6	1.19 (3H, *s*)	C-1, 5, 9, 10, 26	H-1β, 2β, 24, 26
26	17.3	1.30 (3H, *s*)	C-7,8, 9, 14, 25	H-6, 13, 25
27	17.0	0.91 (3H, *s*)	C-8, 13, 14, 15, 28	H-5, 7α, 12α, 15α, 16α, 28
28	16.3	0.77 (3H, *s*)	C-13, 17, 18, 19, 27	H'-19α, 20α, 27, 29, 30
29	28.8	1.18 (3H, *s*)	C-21, 22	H-21, 28
30	30.9	1.21 (3H, *s*)	C-21, 22	H-16β, 21, 28

3. Supplemental data for structure elucidation of compound 3



3a
Divaricatic acid

3b
2-*O*-Methylnordivaricatic acid

Figure S2: The two possible structures of compound **3**

Table S3. ^{1}H and ^{13}C-NMR of compound **3** and divaricatic acid **3a** in acetone-*d6* and MeOD-*d4*

Position	Acetone-*d6*				MeOD-*d4*			
	Compound **3**		Divaricatic acid **3a** [3]		Compound **3**		Divaricatic acid **3a** [4]	
	^{13}C-NMR (125 MHz)	**^{1}H-NMR** (500 MHz)	**^{13}C-NMR** (150 MHz)	**^{1}H-NMR** (600 MHz)	**^{13}C-NMR** (125 MHz)	**^{1}H-NMR** (500 MHz)	**^{13}C-NMR** (100 MHz)	**^{1}H-NMR** (400 MHz)
1	105.7		105.4		107.3		105.5	
2	166.1		166.4		165.6		163.9	
3	99.8	6.39 (1H, *d*, 2.5 Hz)	99.9	6.41 (1H, *d*, 2.5 Hz)	100.1	6.40 (1H, *d*, 2.5 Hz)	98.54	6.30 (1H, *s*)
4	165.3		165.6		165.4		163.7	
5	111.3	6.45 (1H, *d*, 2.5 Hz)	111.6	6.46 (1H, *d*, 2.5 Hz)	111.2	6.42 (1H, *d*, 2.5 Hz)	109.4	6.08 (1H, *s*)
6	148.4		148.6*		148.4		146.8	
7	170.2		169.2		170.5		171.5	
8	39.3	2.95 (2H, overlap)	39.3*	2.93-3.00 (2H, *m*)	39.5	2.93 (2H, *t*, 7.5 Hz)	38.0	2.79 (2H, *t*, 7.6 Hz)
9	25.9	1.70 (2H, *m*)	25.7*	1.61-1.77 (2H, *m*)	26.4	1.65-1.73 (2H, *m*)	24.7	1.57 (2H, *m*)
10	14.4	0.95 (3H, *t*, 7.0Hz)	14.4*	0.93-1.00 (3H, *m*)	14.5	0.99 (3H, *t*, 7.5 Hz)	13.3	0.94 (3H, *t*, 7.2 Hz)
1′	116.2		112.2		116.9		110.1	
2′	165.3		165.2		164.5		157.9	
3′	114.5	6.57 (1H, *d*, 2.5Hz)	109.3	6.79 (1H, *d*, 2.3 Hz)	115.2	6.54 (1H, *d*, 2.0 Hz)	99.8	6.08 (1H, *d*, 2,4 Hz)
4′	152.8		155.0		153.4		159.3	
5′	108.2	6.50 (1H, *d*, 2.5Hz)	116.6	6.77 (1H, *d*, 2.3 Hz)	108.3	6.49 (1H, *d*, 2.5 Hz)	108.8	6.08 (1H, *d*, 2,4 Hz)
6′	149.3		149.1*		149.5		148.0	
7′	176.7		173.3		#		175.12	
8′	38.0	3.12 (2H, *t*, 7.5 Hz)	38.7*	2.93-3.00 (2H, *m*)	38.4	3.14 (2H, *t*, 7.5 Hz)	37.31	3.00 (2H, *t*, 7.6 Hz)
9′	25.5	1.63 (2H, *sextet*, 7.5 Hz)	25.9*	1.61-1.77 (2H, *m*)	26.1	1.65-1.73 (2H, *m*)	24.7	1.57 (2H, *m*)
10′	14.4	0.90 (3H, *t*, 7.0Hz)	14.5*	0.93-1.00 (3H, *m*)	14.6	0.98 (3H, *t*, 6.5 Hz)	13.1	0.93 (3H, *t*, 7.2 Hz)
2–OH	-	11.17 (1H, *s*)	-					
2′–OCH$_3$	55.9	3.86 (3H, *s*)	-	-	55.9	3.84 (3H, *s*)	-	-
4–OCH$_3$	-	-	55.9	3.86 (3H, *s*)			54.4	3.78 (3H, *s*)
–COOH		14.22 (1H, *brs*)						

* These signals were interchangeable # This signals was not observed in ^{13}C-NMR

Reference

[1] T. T. Nguyen, Q. C. T. Nguyen, V. H. Mai, Q. T. Phan, P. Q. Do, T. D. Nguyen, P. D. Nguyen, T. T. T. Nguyen, T. D. Le, T. X. T. Dai, K. Kamei and K. Kanaori (2019). A new hopane derivative from the lichen *Dirinaria applanata*, *Nat. Prod. Res.*, 1-5.

[2] J. Sichaem, H. H. Nguyen and T. H. Duong (2019). Hopane-6*α*,16*α*,22-triol: A New Hopane Triterpenoid from the Lichen *Parmotrema sancti-angelii*, *Nat. Prod. Commun.* **14**, 1934578X19858208.

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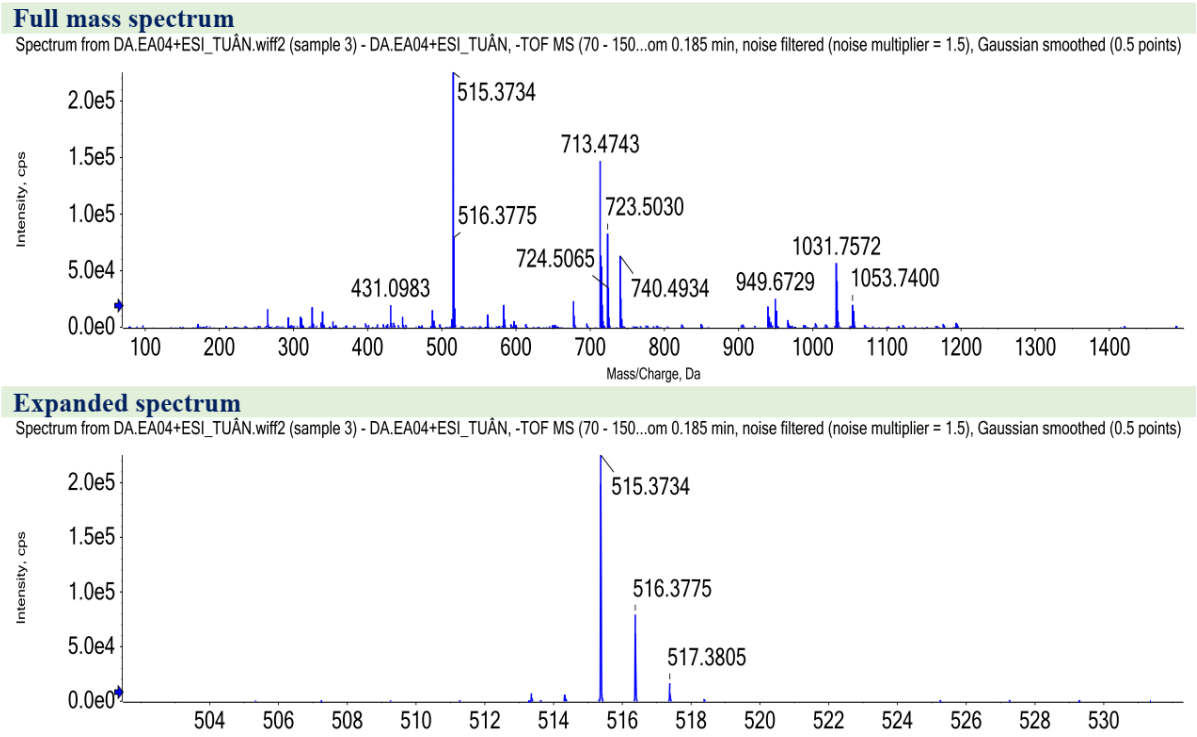
[4] P. Kumboonma, T. Senawong, S. Saenglee, C. Yenjai and C. Phaosiri (2018). New histone deacetylase inhibitors from the twigs of *Melanorrhoea usitata*, *Med. Chem. Res.* **27**, 2004-2015.

4. Scanned spectra of all compounds



Formula (M)	Ion formula	*m/z*	Calcd *m/z*	Diff (ppm)
$C_{32}H_{52}O_5$	$C_{32}H_{51}O_5^-$	515.3734	515.3736	0.39 ppm

Figure S3: (–)HRESI-MS of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

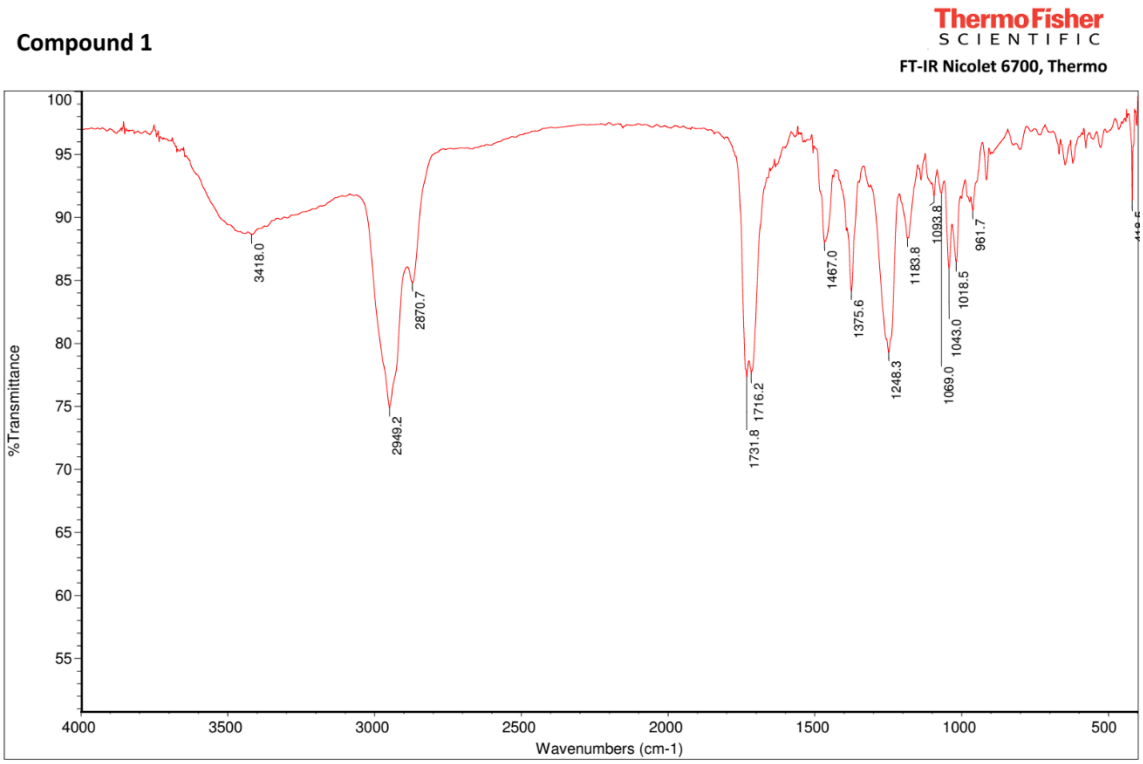


Figure S4: FT-IR of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

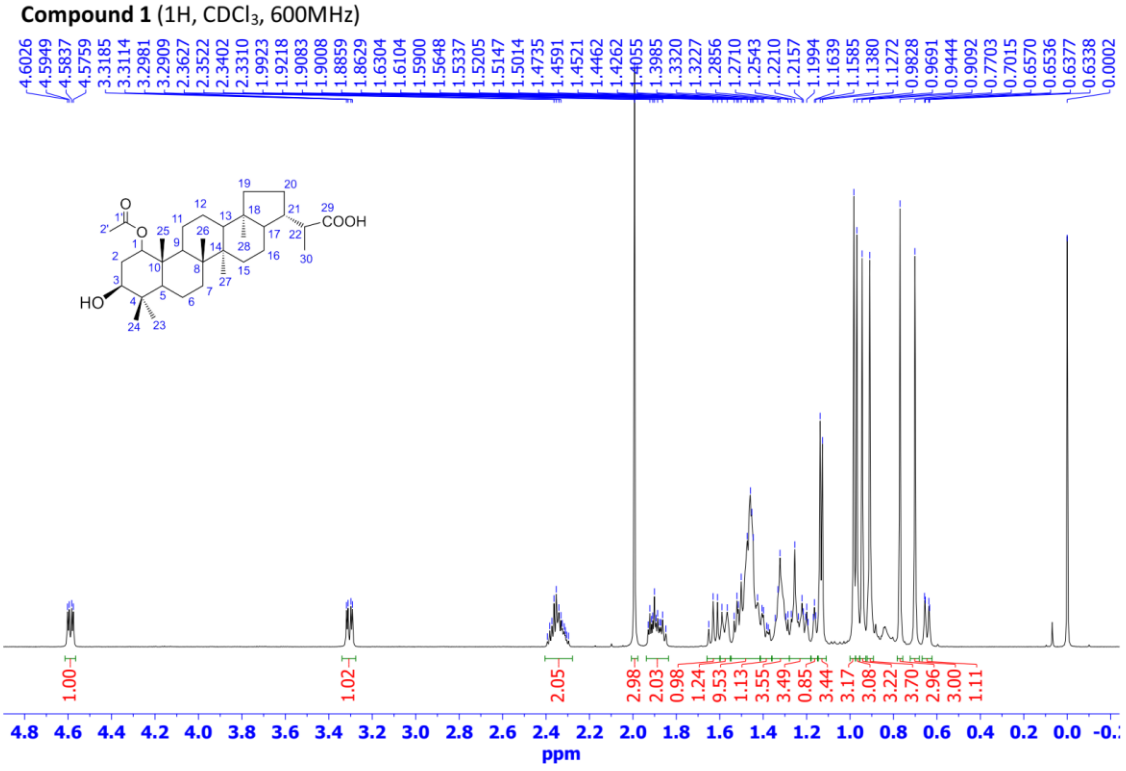


Figure S5: Full ^{1}H-NMR of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

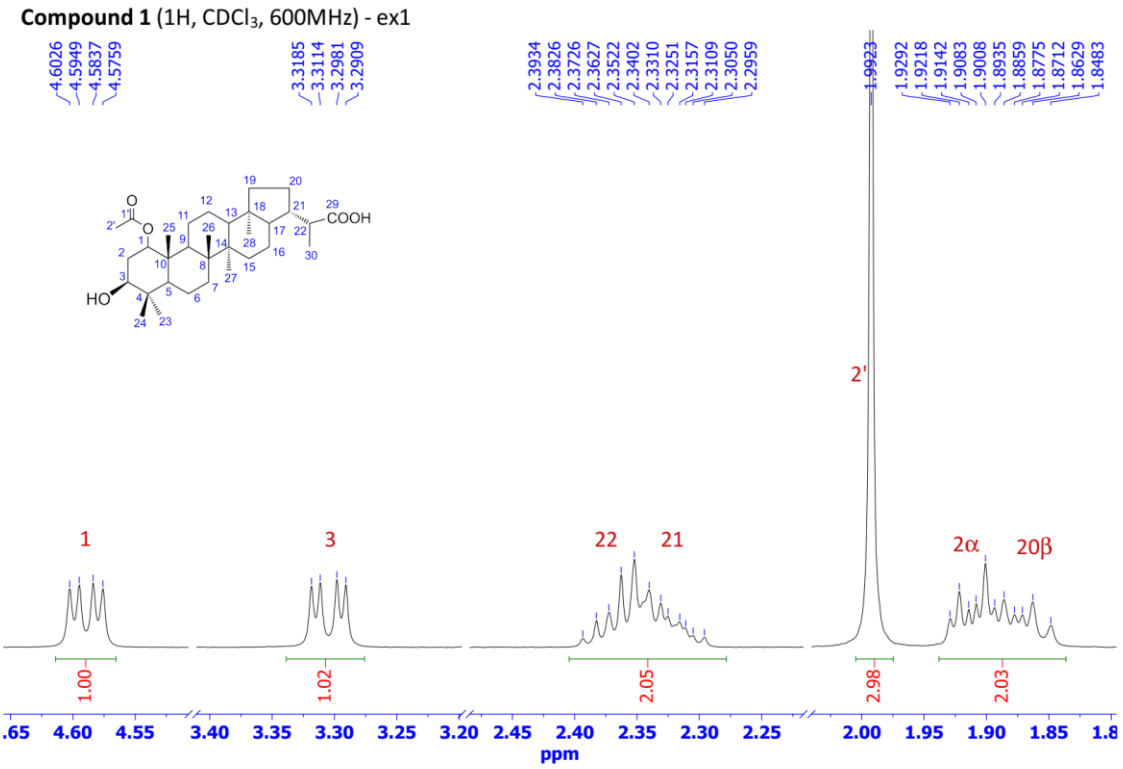


Figure S6: Extended ^{1}H-NMR of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

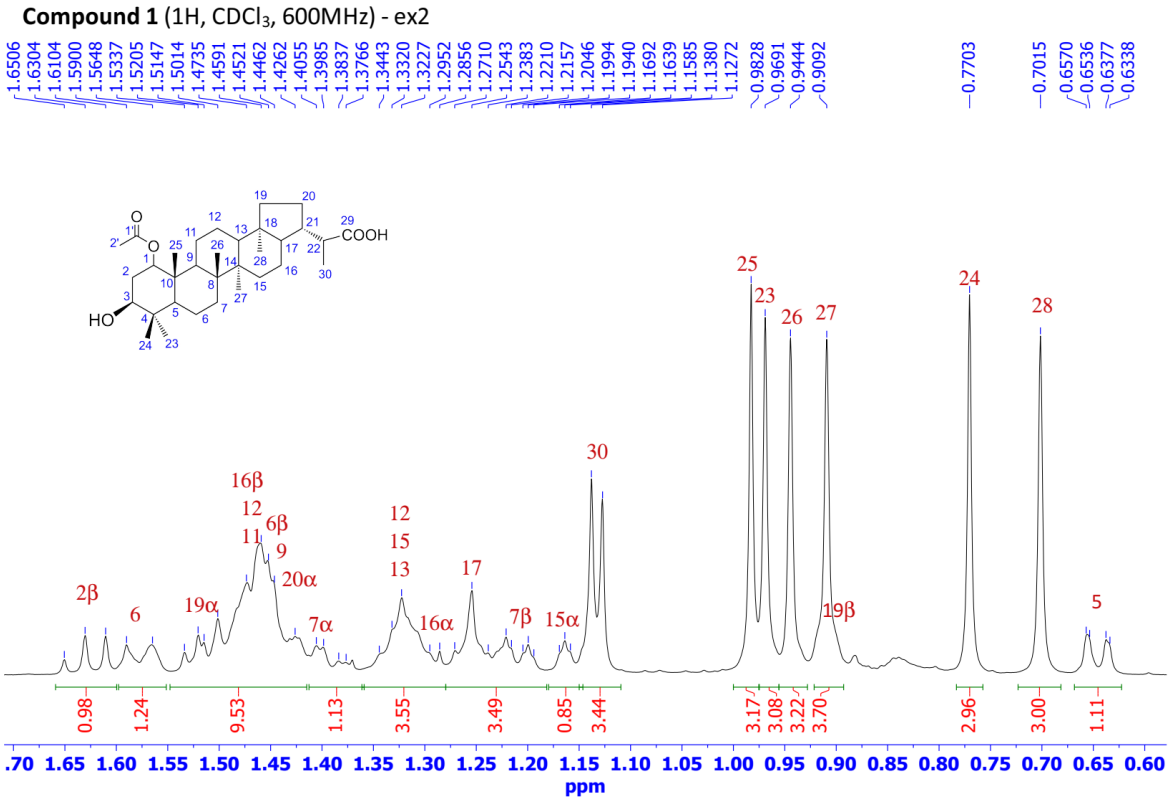


Figure S7: Extended ^{1}H-NMR of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

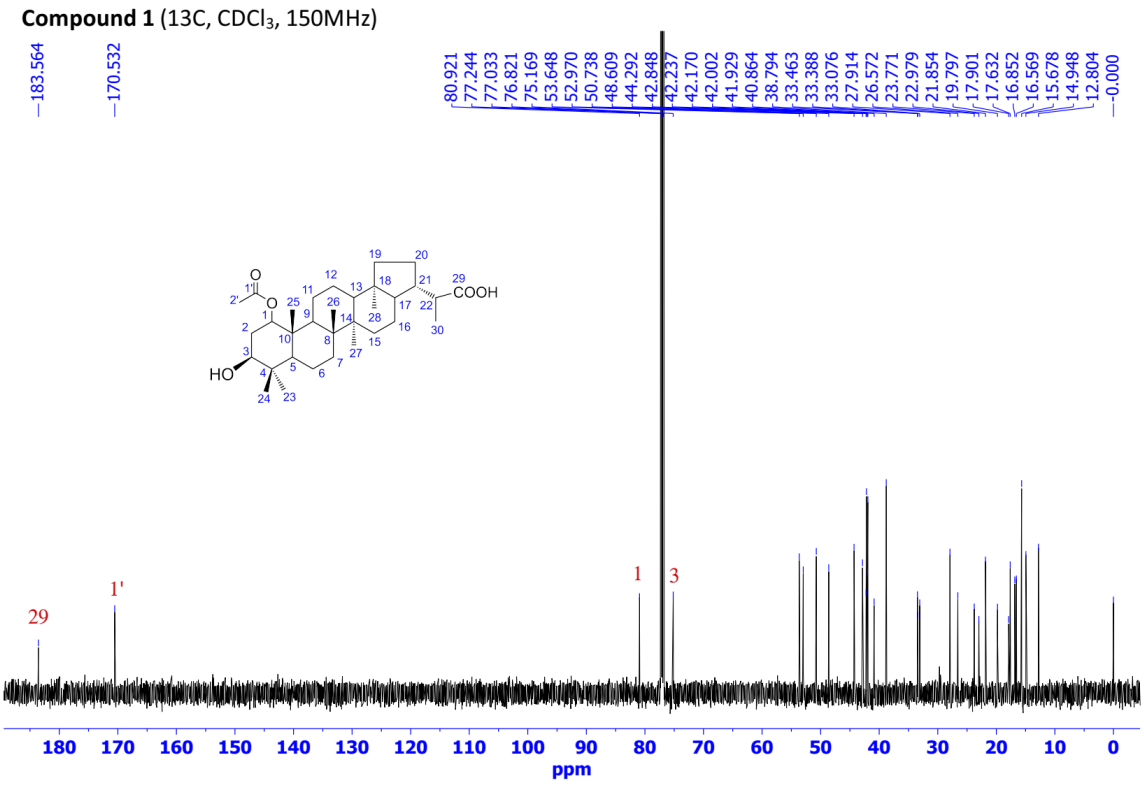


Figure S8: Full ^{13}C-NMR of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

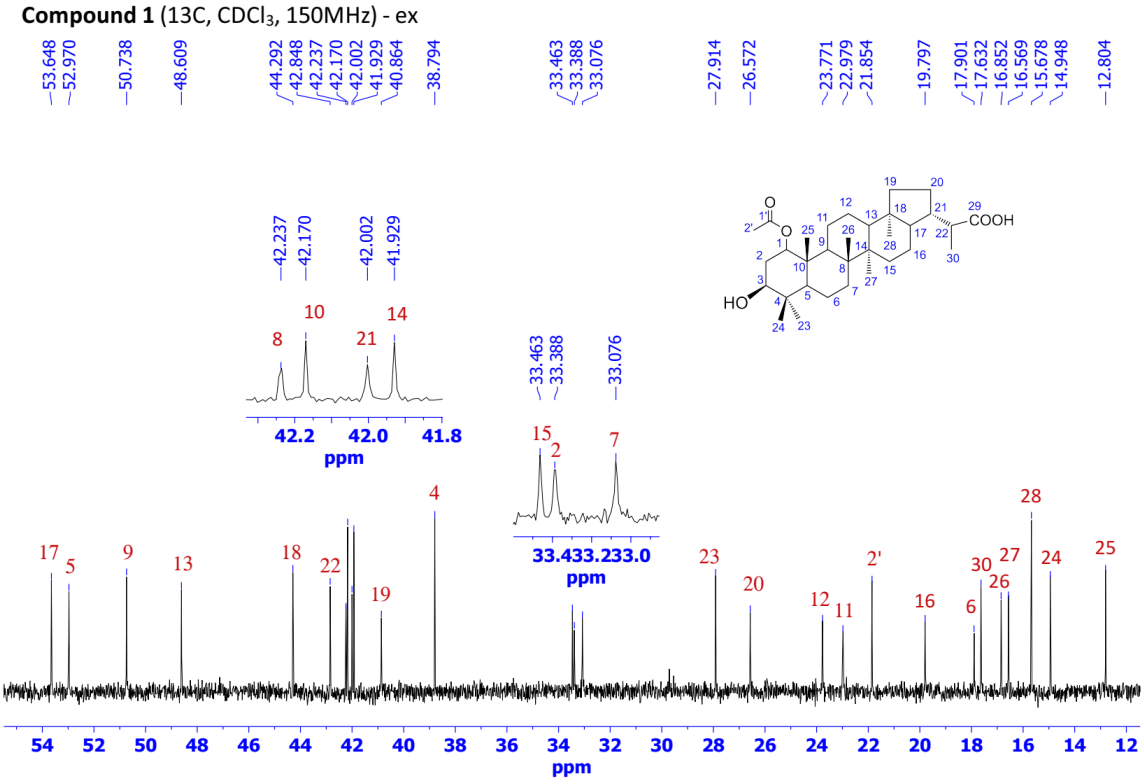


Figure S9: Extended ^{13}C-NMR of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

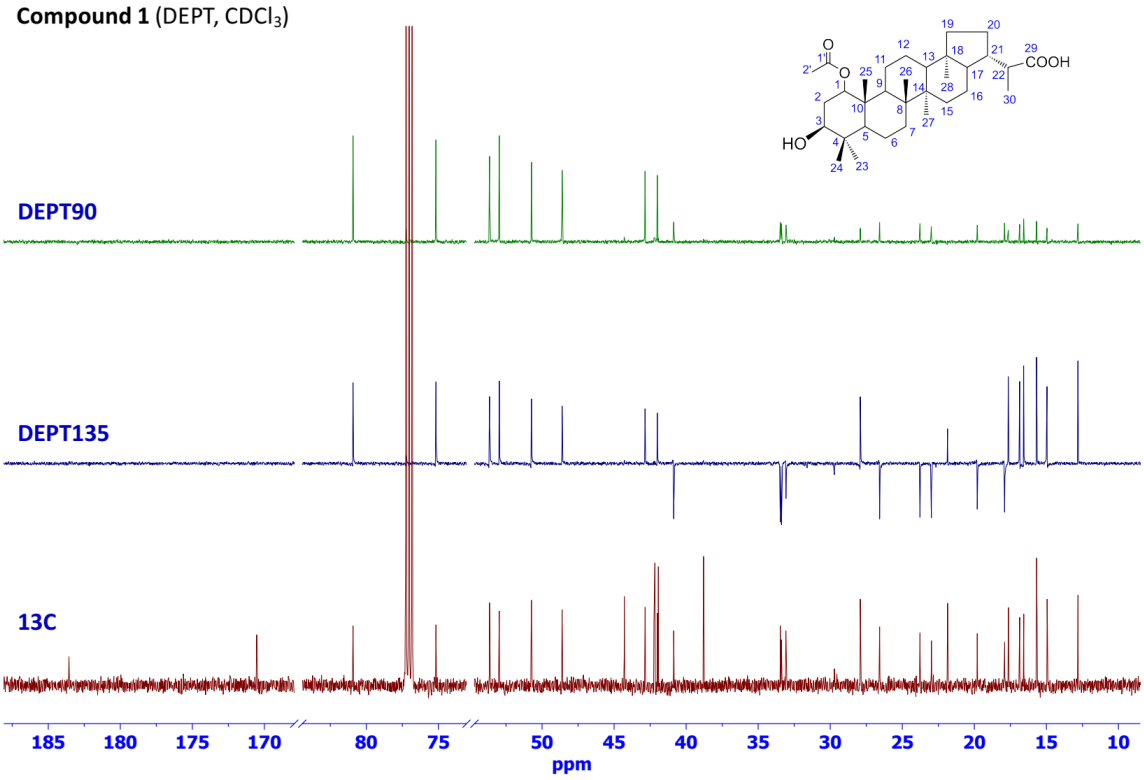


Figure S10: Full DEPT of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

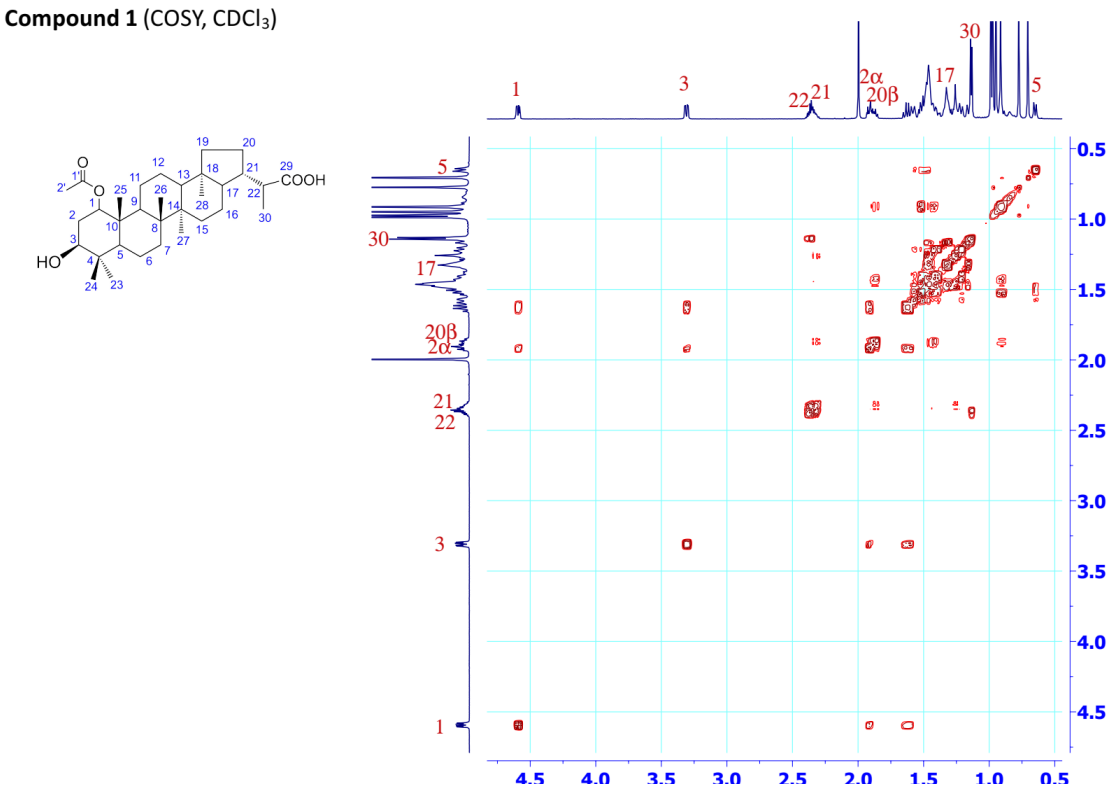


Figure S11: Full COSY of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

Compound 1 (HSQC, $CDCl_3$)

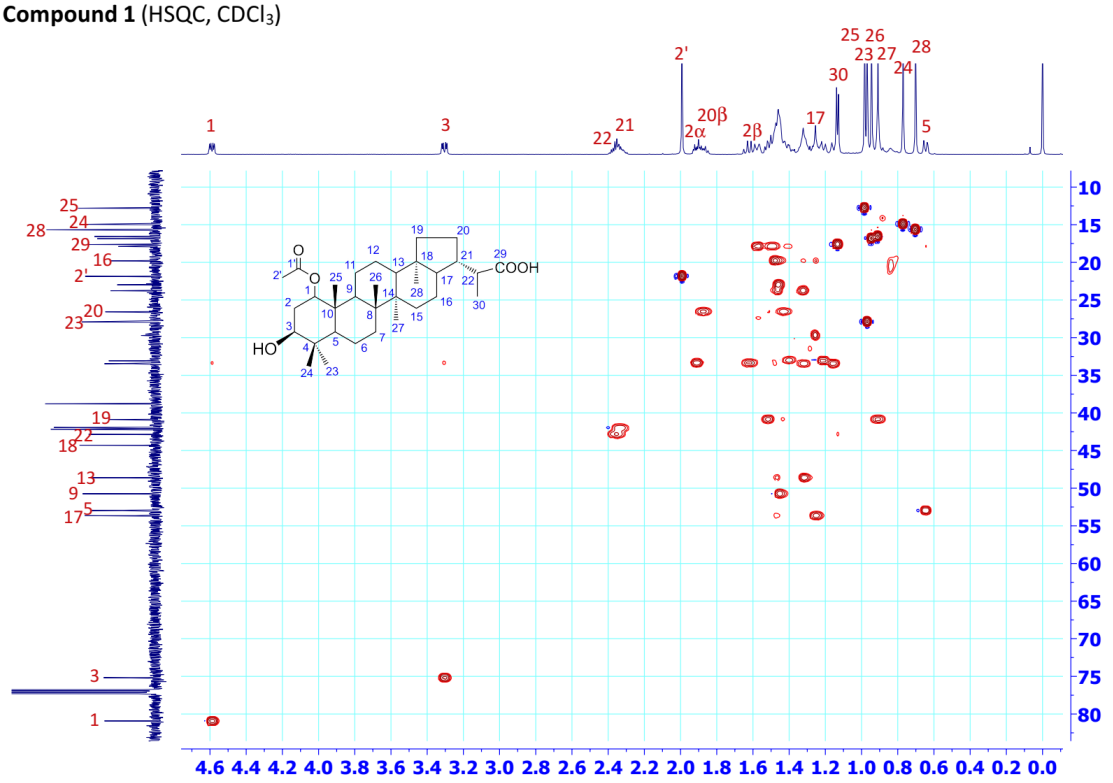


Figure S12: Full HSQC of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

Compound 1 (HMBC) - ex1

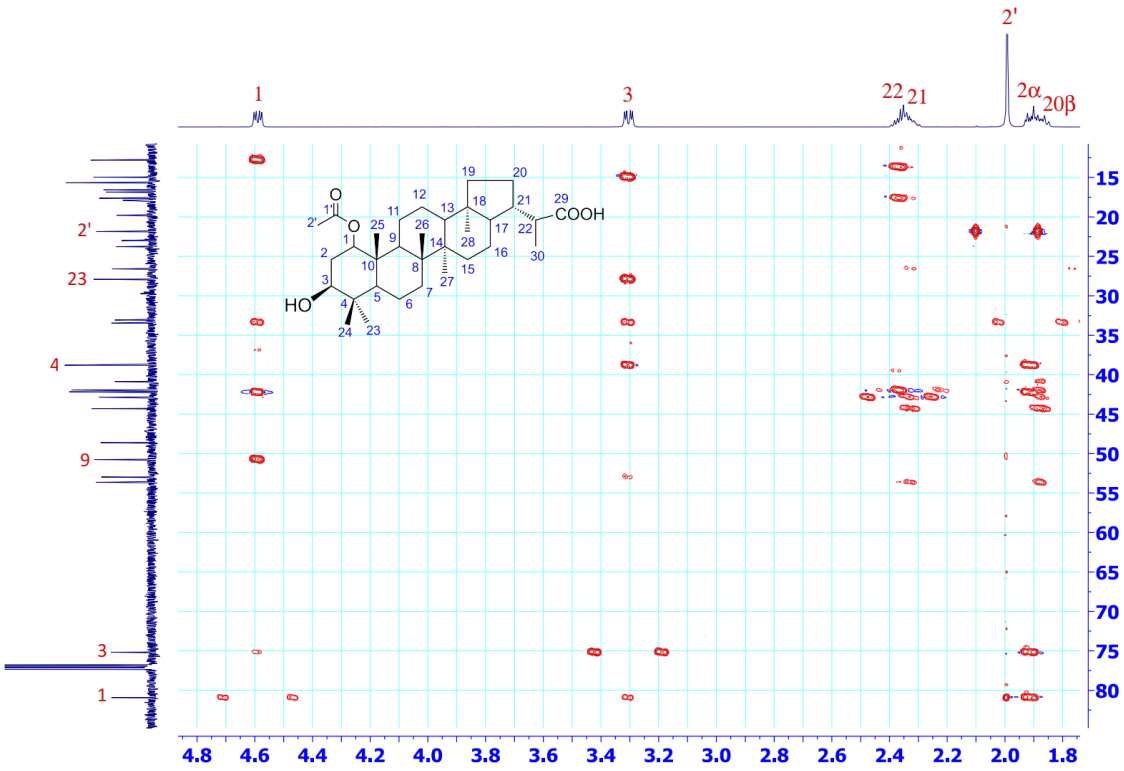


Figure S13: Extended HMBC of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

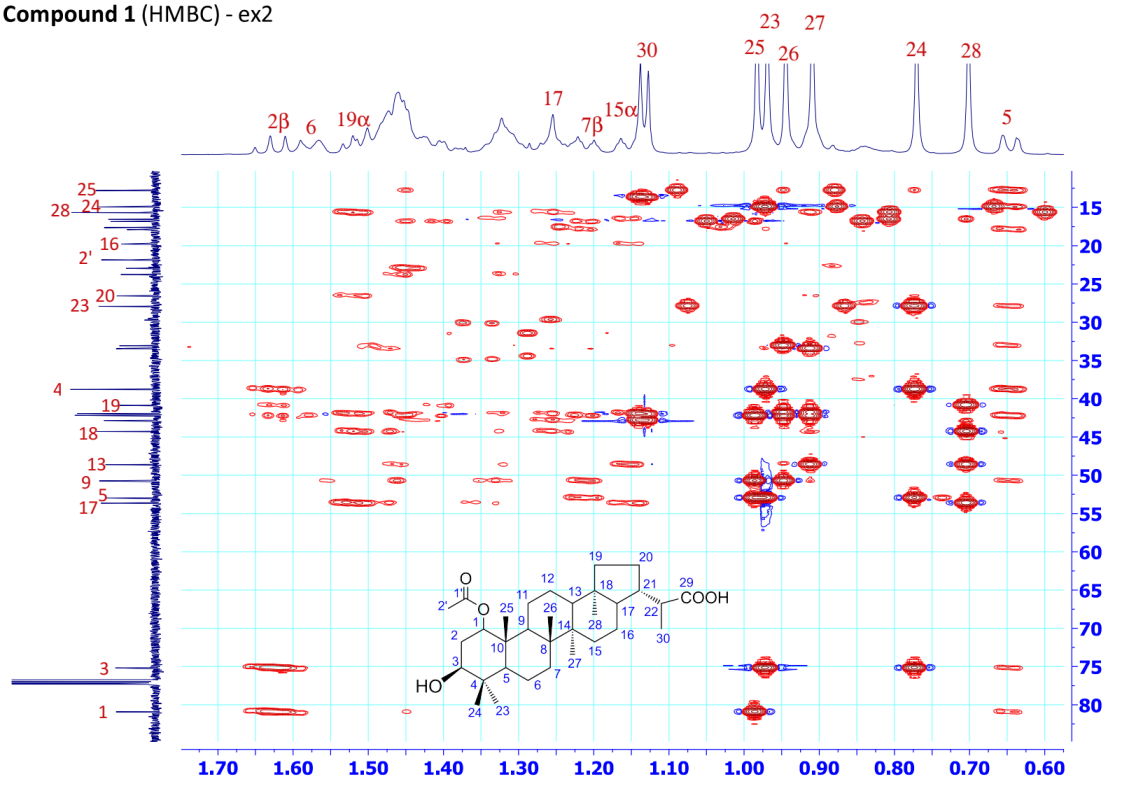


Figure S14: Extended HMBC of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

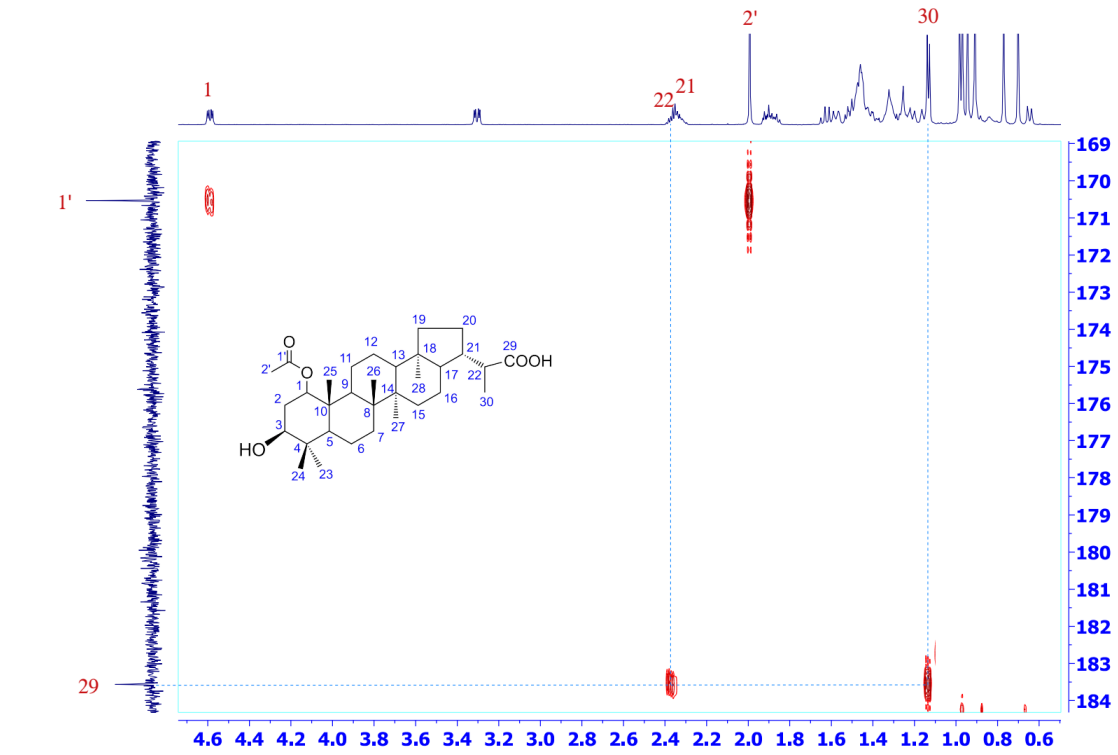


Figure S15: Extended HMBC of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

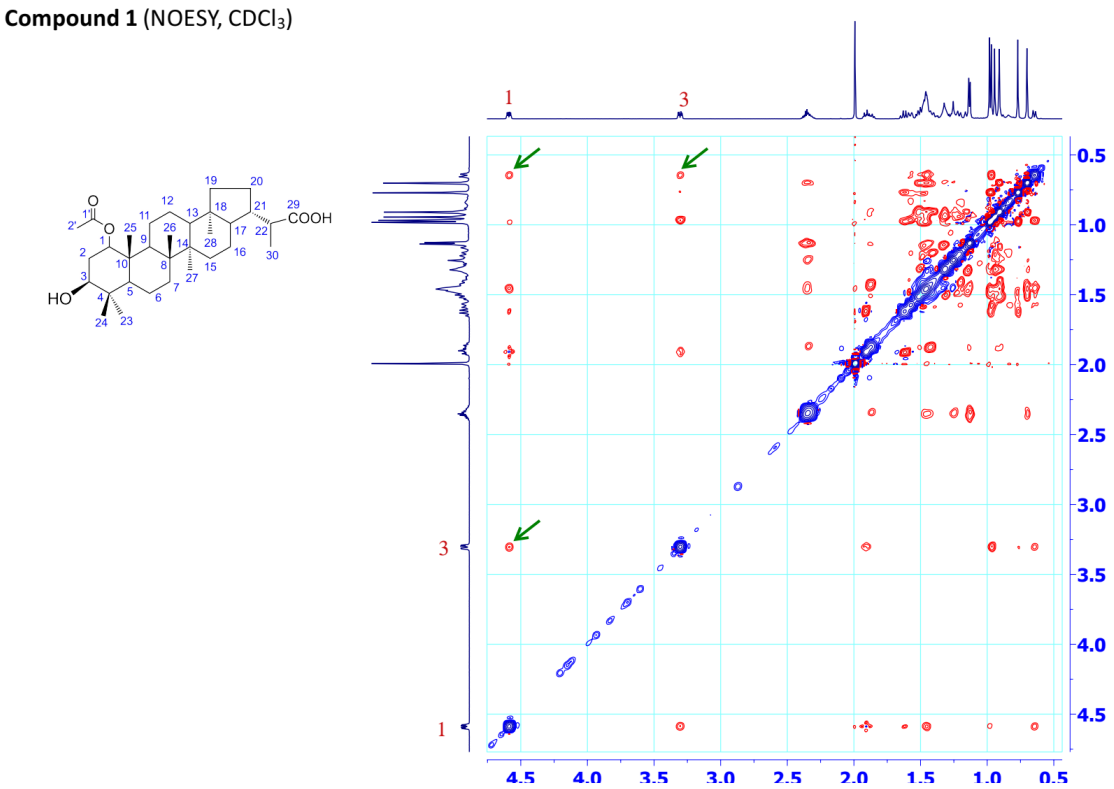


Figure S16: Full NOESY of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

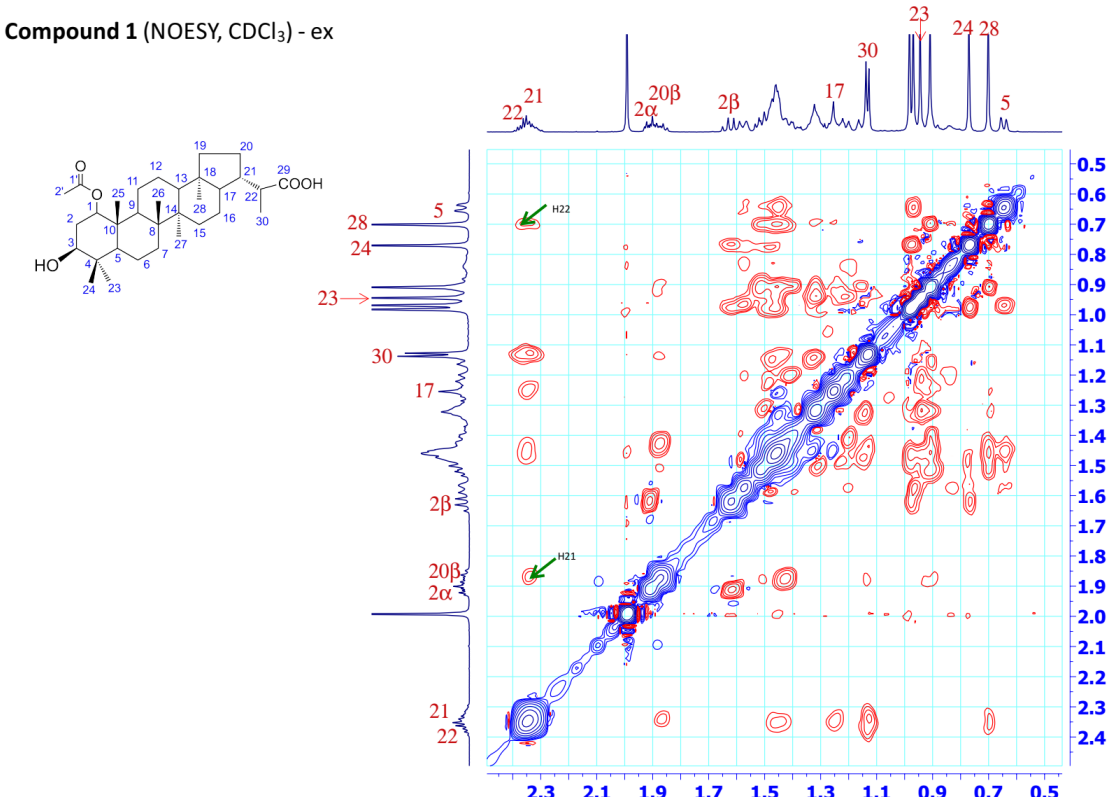
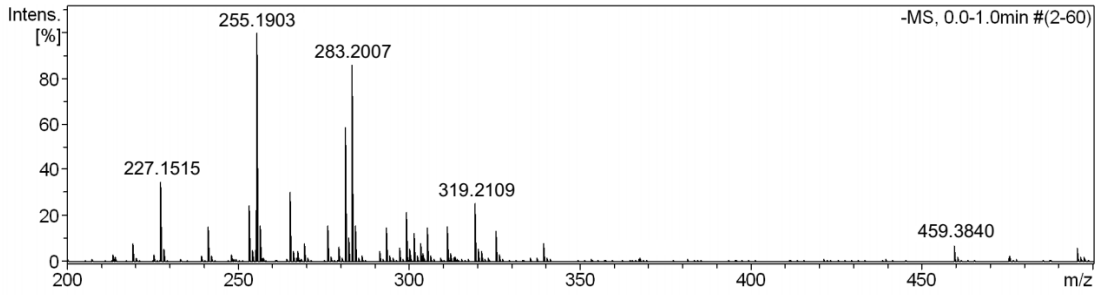


Figure S17: Extended NOESY of compound **1** (1β-acetoxy-3β-hydroxy-21α-hopan-29-oic acid)

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	200 m/z	Set Capillary	4500 V	Set Dry Gas	5.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Formula (M)	Ion formula	*m/z*	Calcd *m/z*	Diff (ppm)
$C_{30}H_{52}O_3$	$C_{30}H_{51}O_3$	459.3840	459.3843	0.65

Figure S18: (–)HRESI-MS of compound **2** (21α-hopane-3β,6β,22-triol)

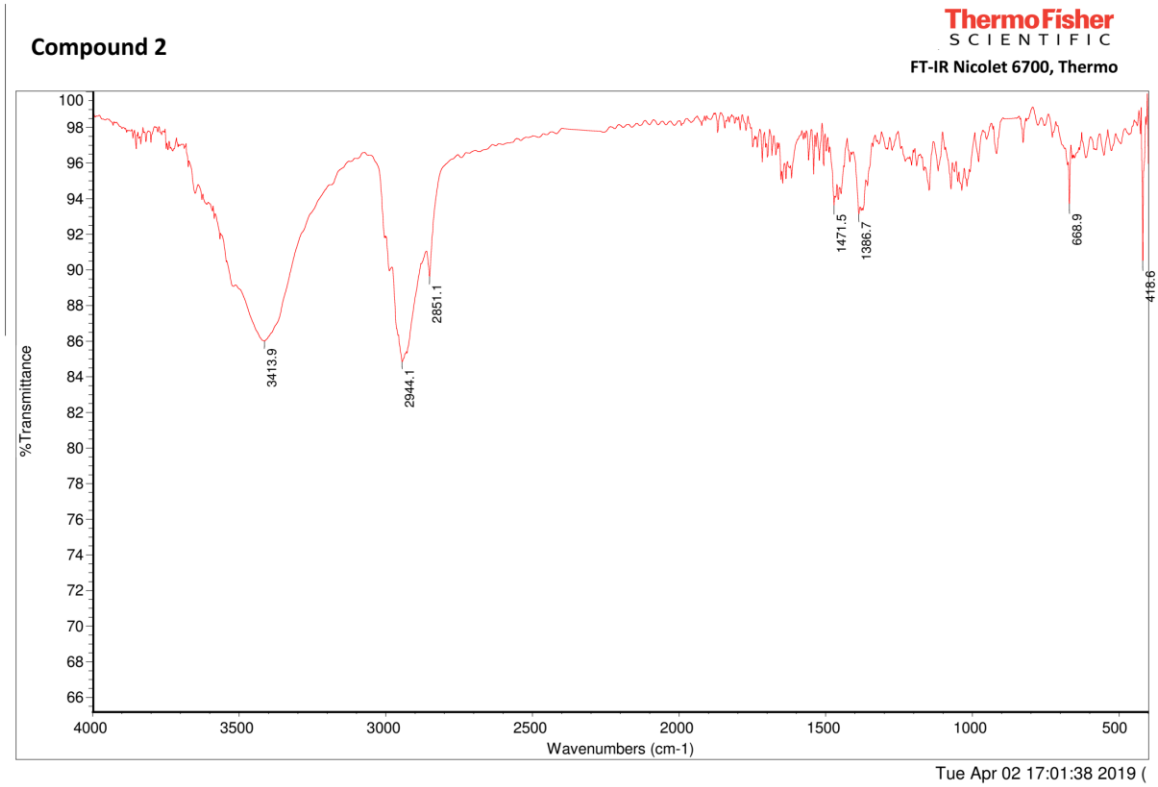


Figure S19: FT-IR of compound **2** (21α-hopane-3β,6β,22-triol)

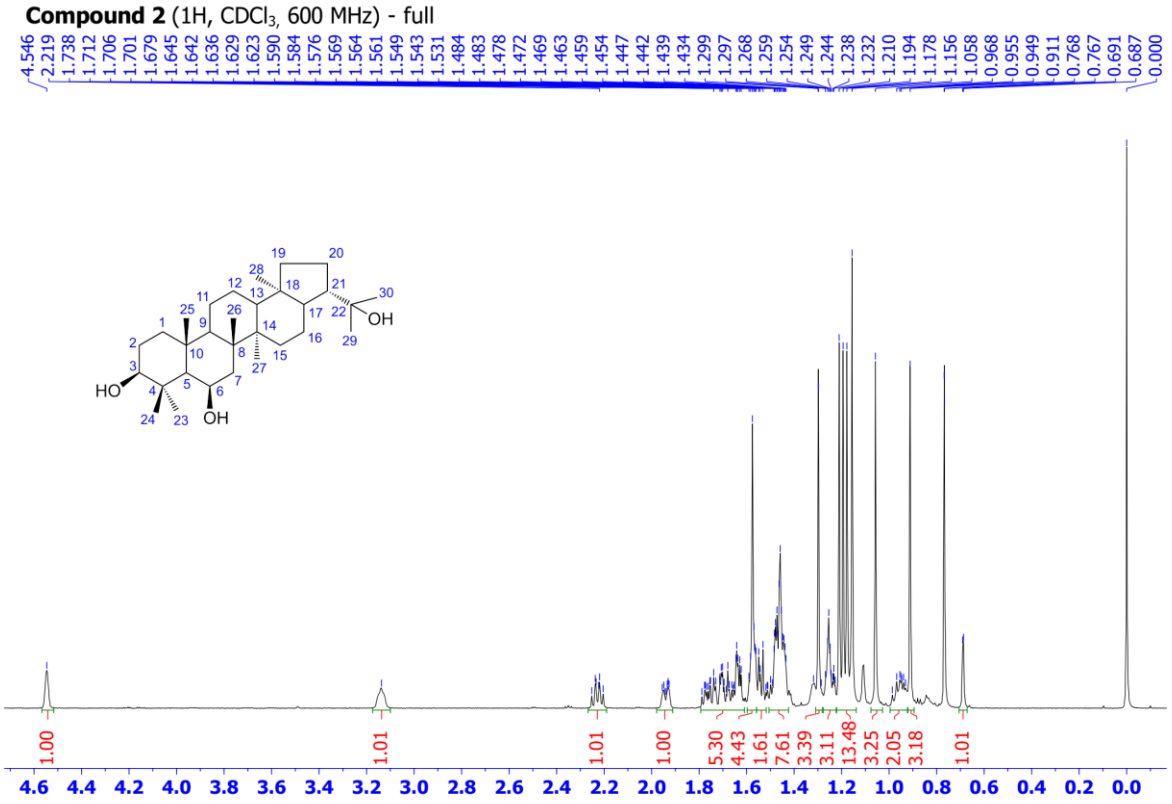


Figure S20: Full ^{1}H-NMR of compound **2** (21α-hopane-3β,6β,22-triol)

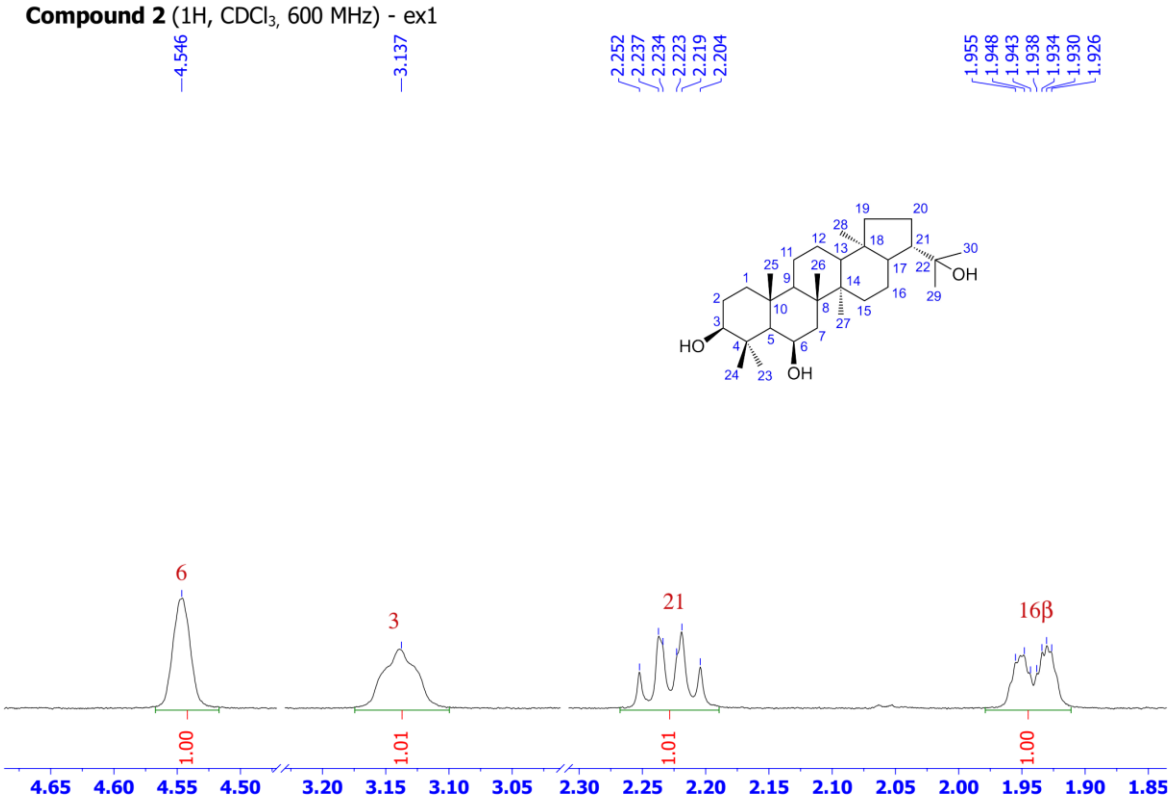


Figure S21: Extended ^{1}H-NMR of compound **2** (21α-hopane-3β,6β,22-triol)

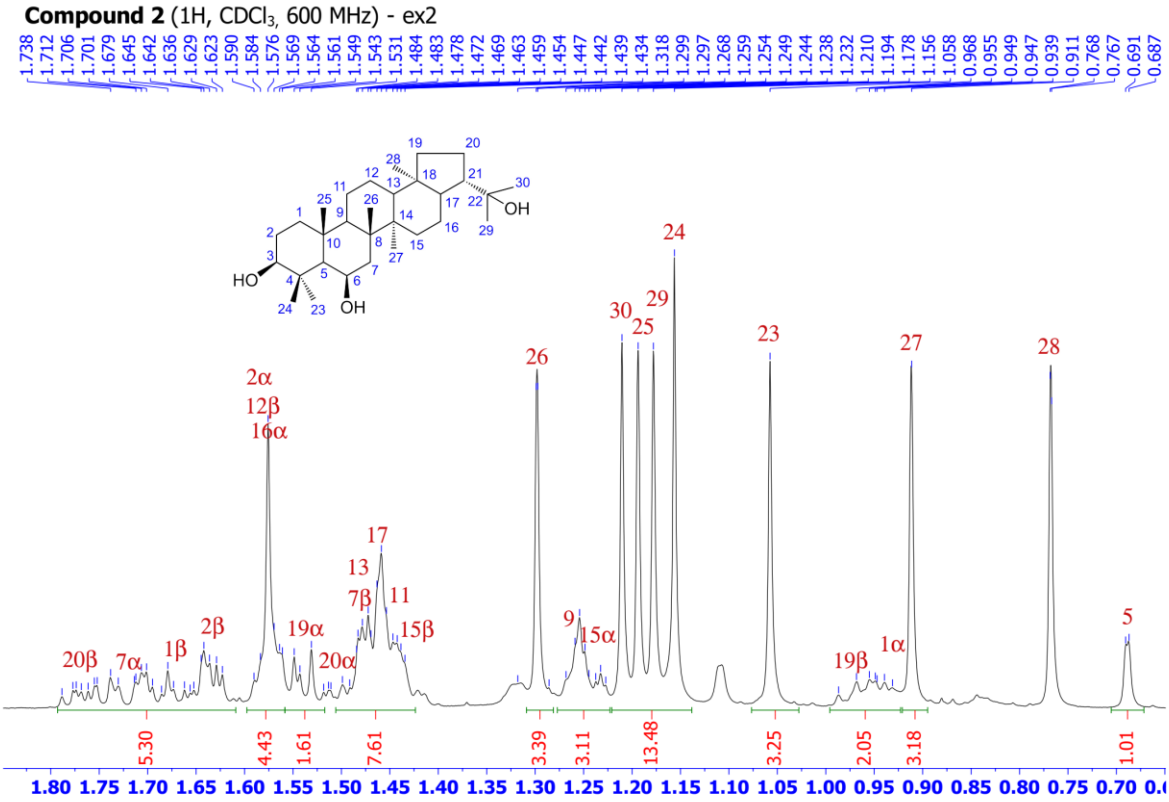


Figure S22: Extended ^{1}H-NMR of compound **2** (21α-hopane-3β,6β,22-triol)

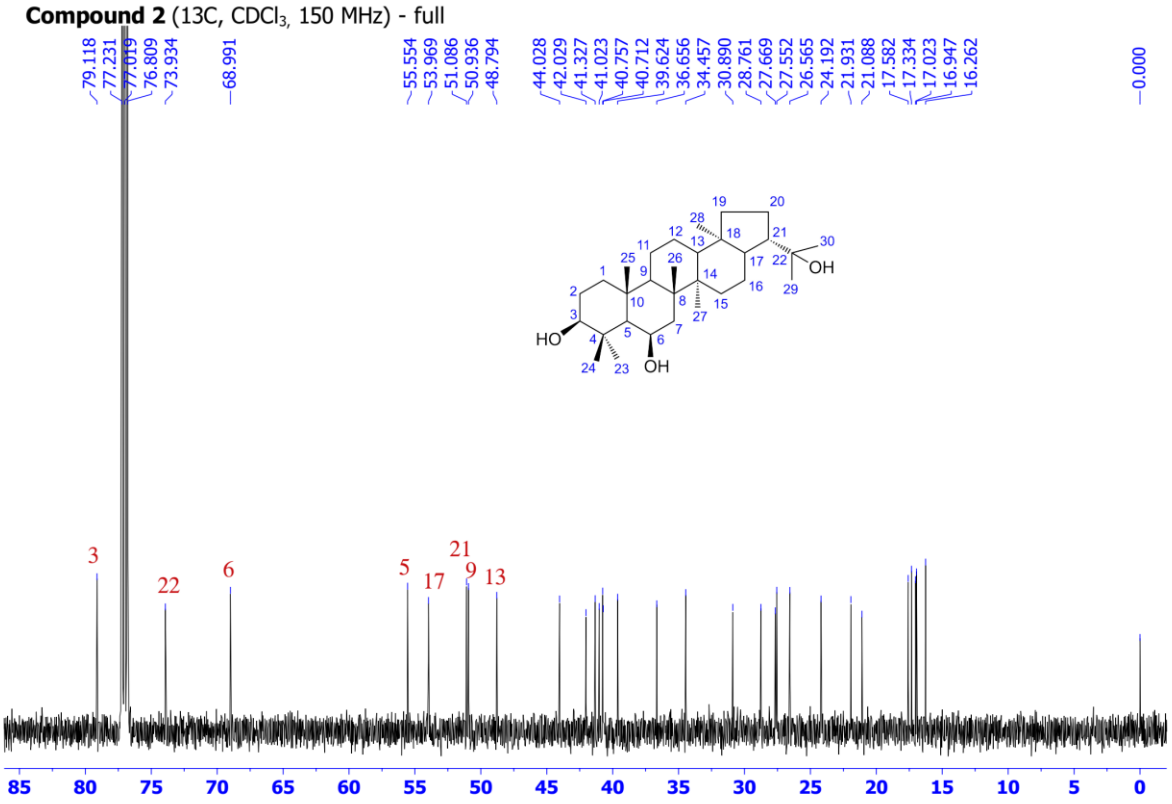


Figure S23: Full ^{13}C-NMR of compound **2** (21α-hopane-3β,6β,22-triol)

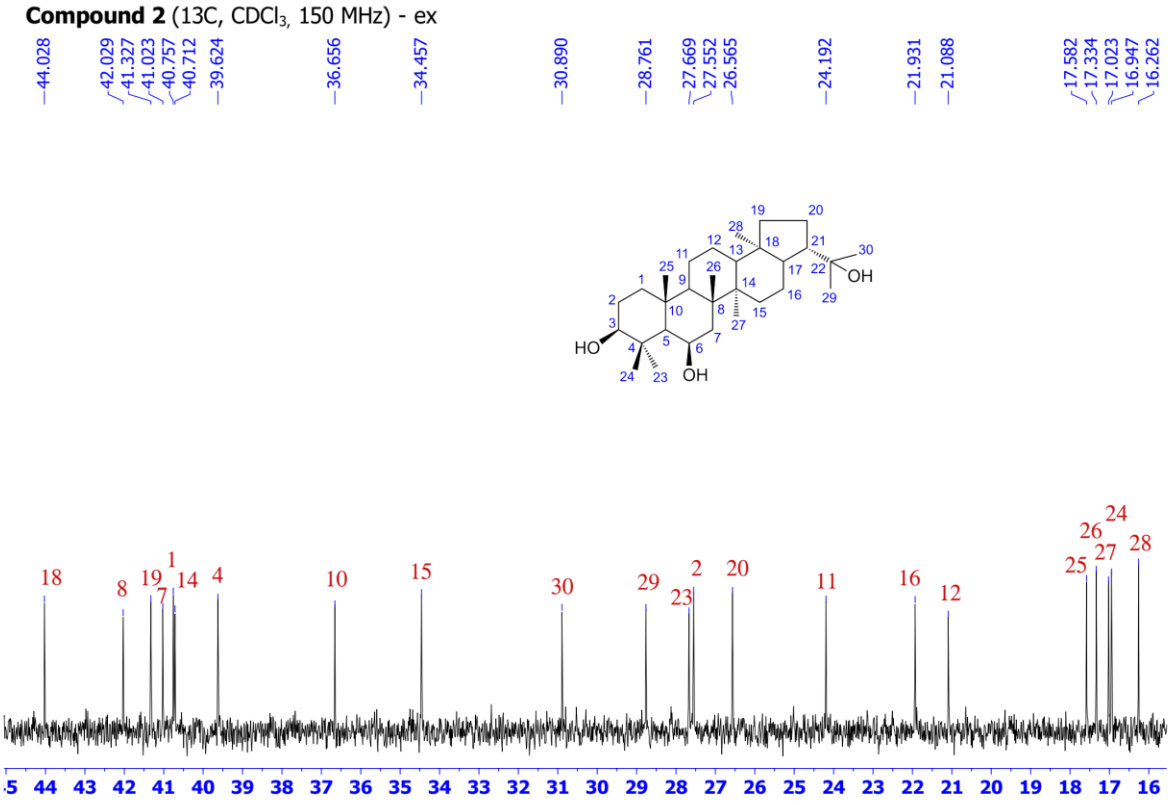


Figure S24: Extended ^{13}C-NMR of compound **2** (21α-hopane-3β,6β,22-triol)

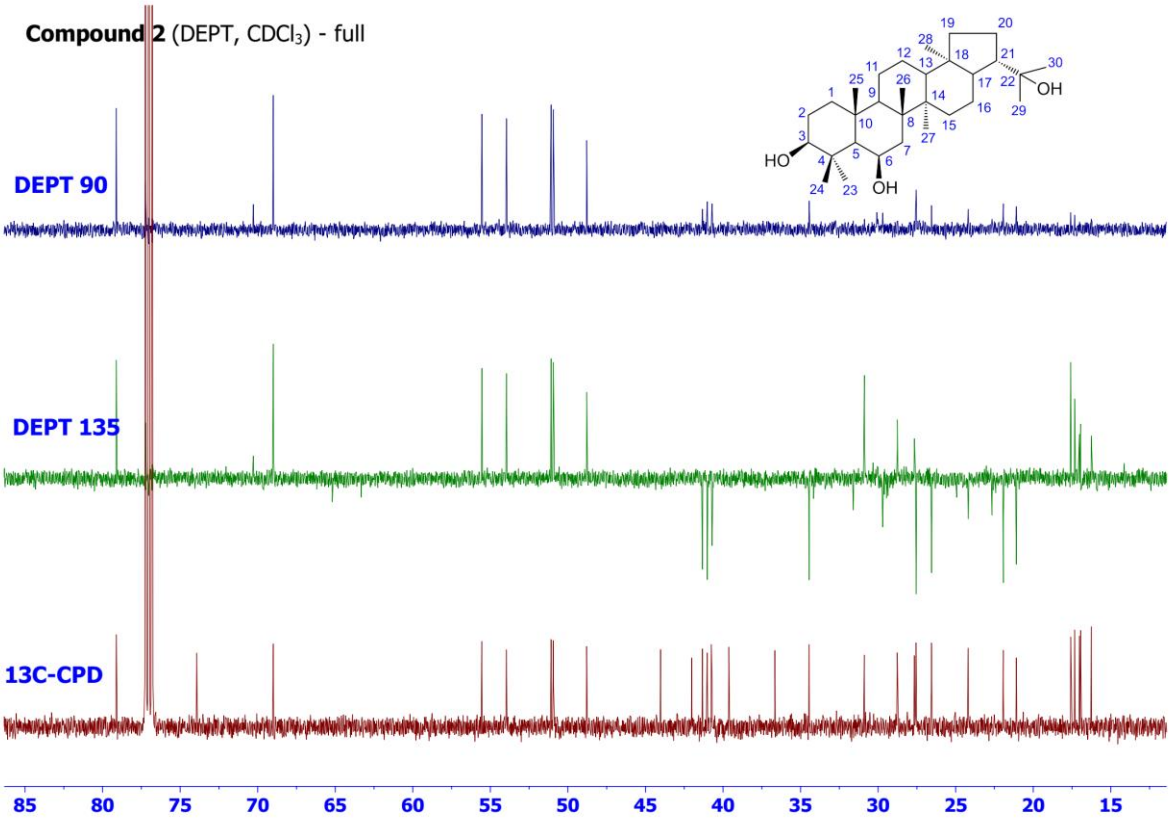


Figure S25: Full DEPT of compound **2** (21α-hopane-3β,6β,22-triol)

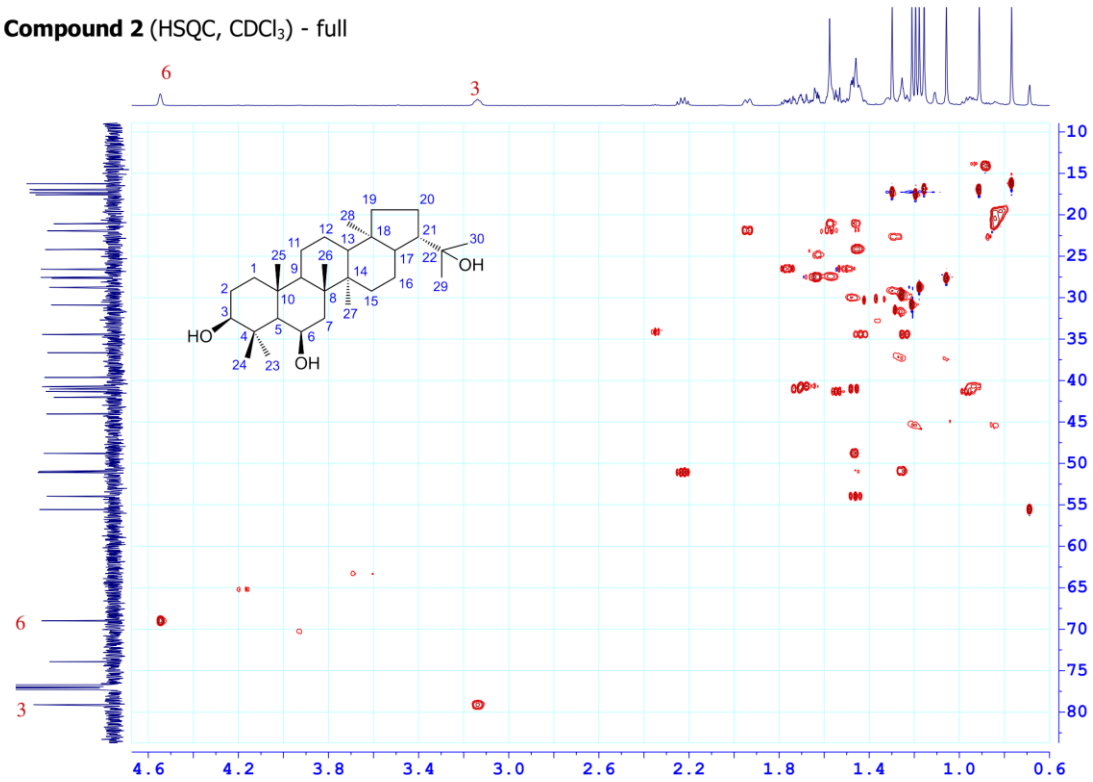


Figure S26: Full HSQC of compound **2** (21α-hopane-3β,6β,22-triol)

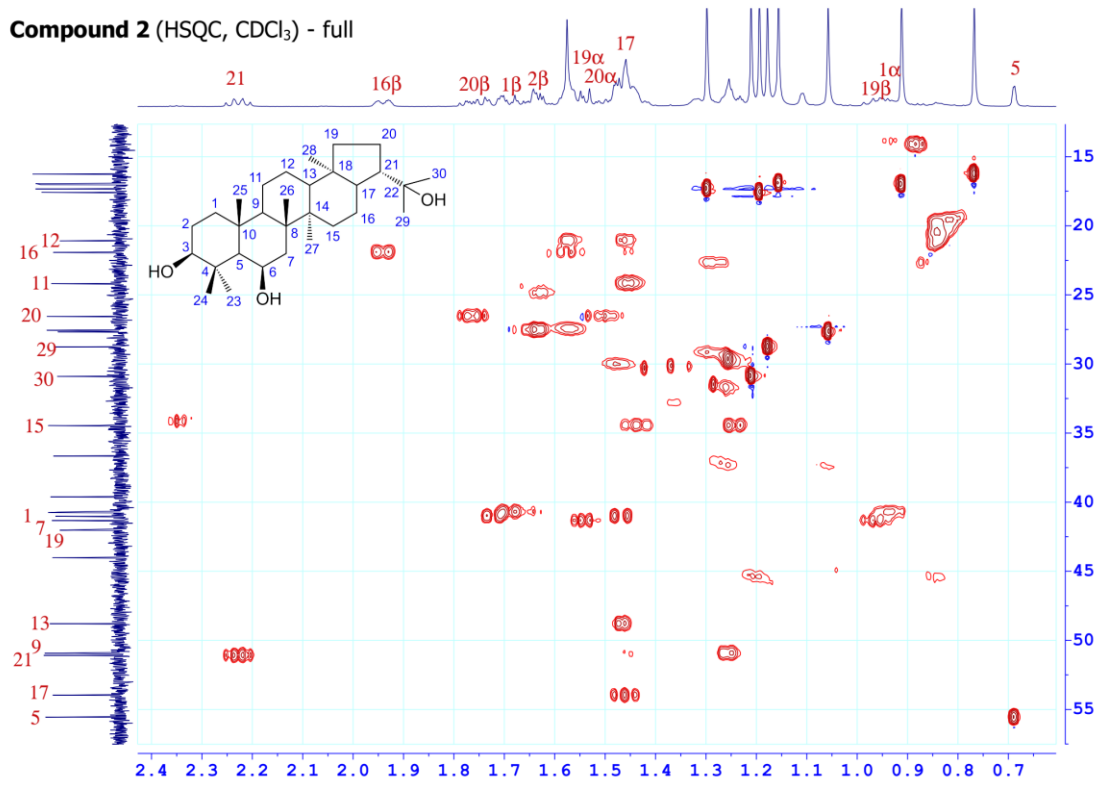


Figure S27: Extended HSQC of compound **2** (21α-hopane-3β,6β,22-triol)

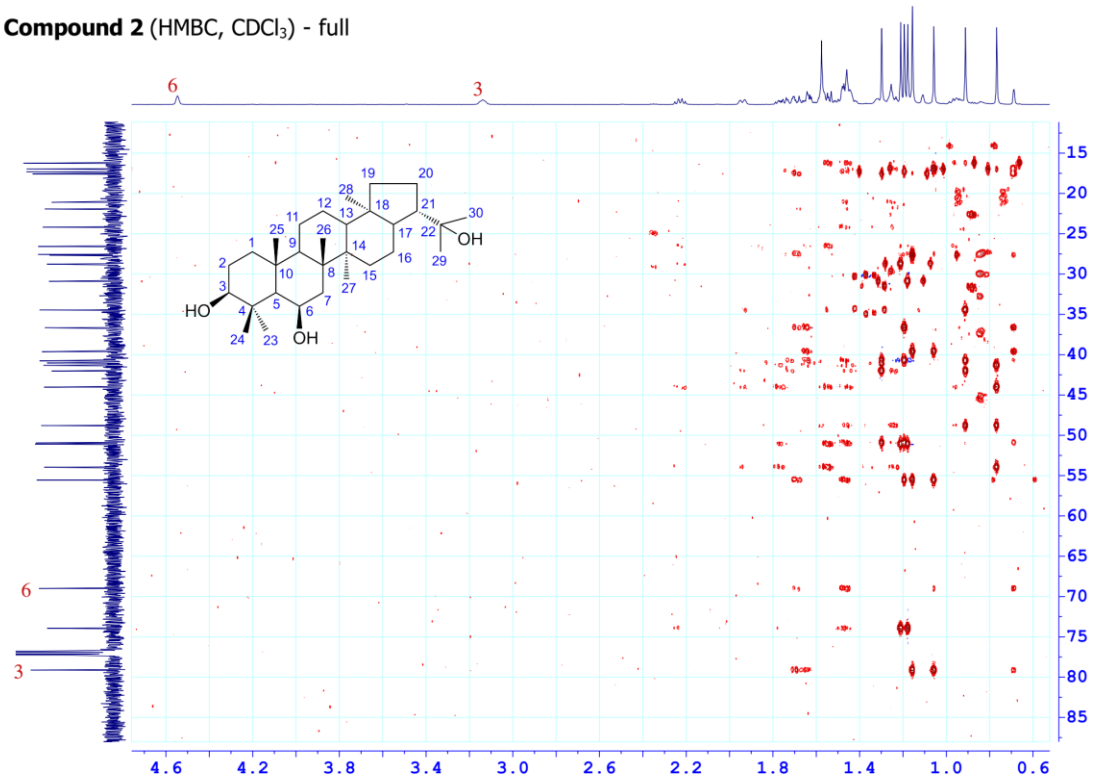


Figure S28: Full HMBC of compound **2** (21α-hopane-3β,6β,22-triol)

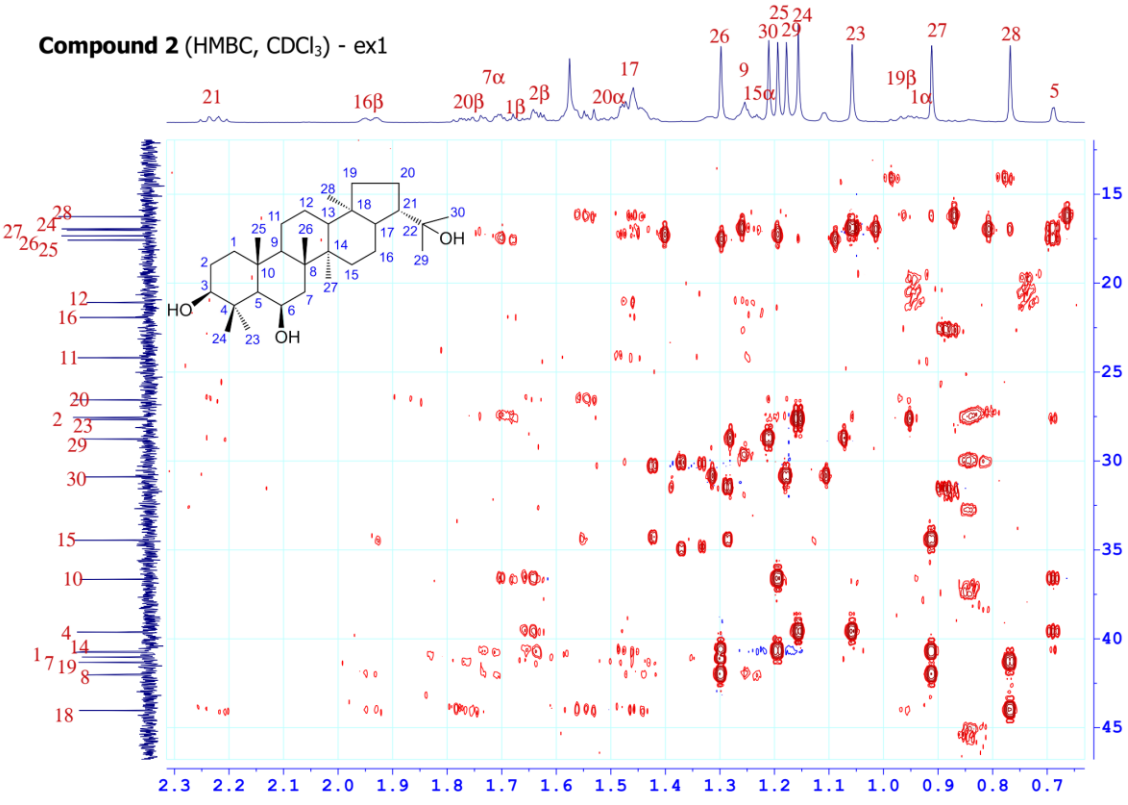


Figure S29: Extended HMBC of compound **2** (21α-hopane-3β,6β,22-triol)

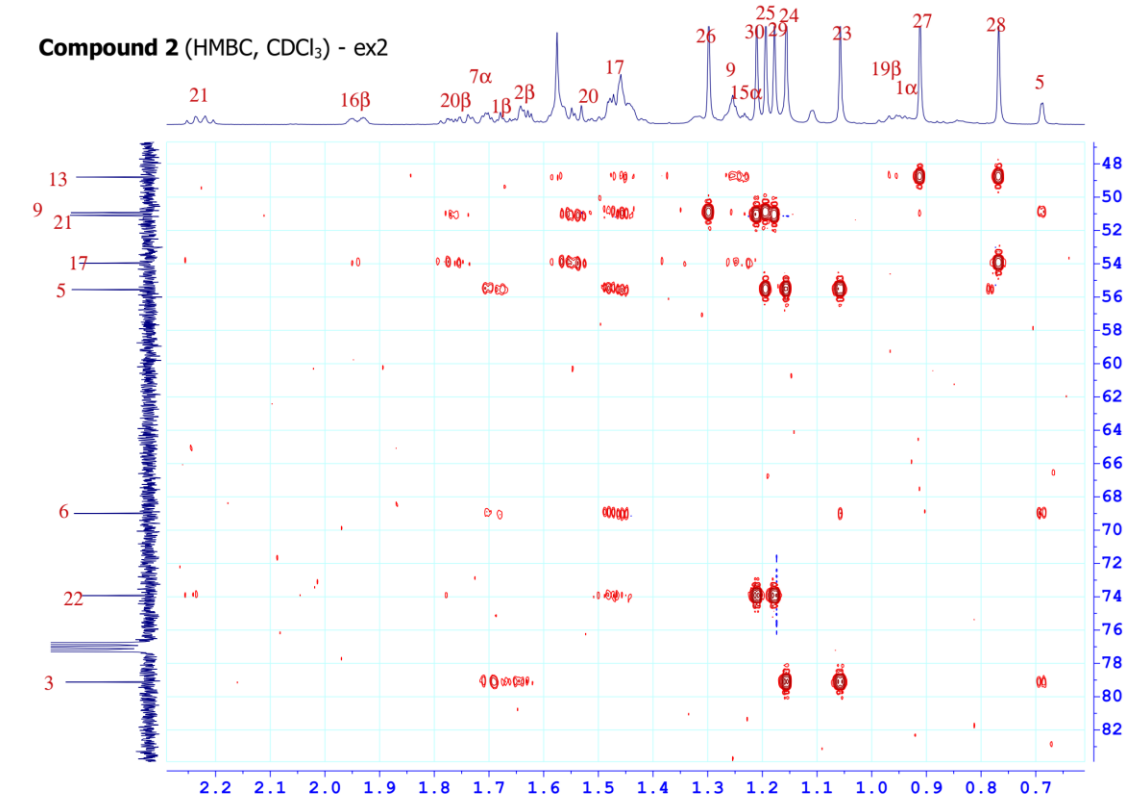


Figure S30: Extended HMBC of compound **2** (21α-hopane-3β,6β,22-triol)

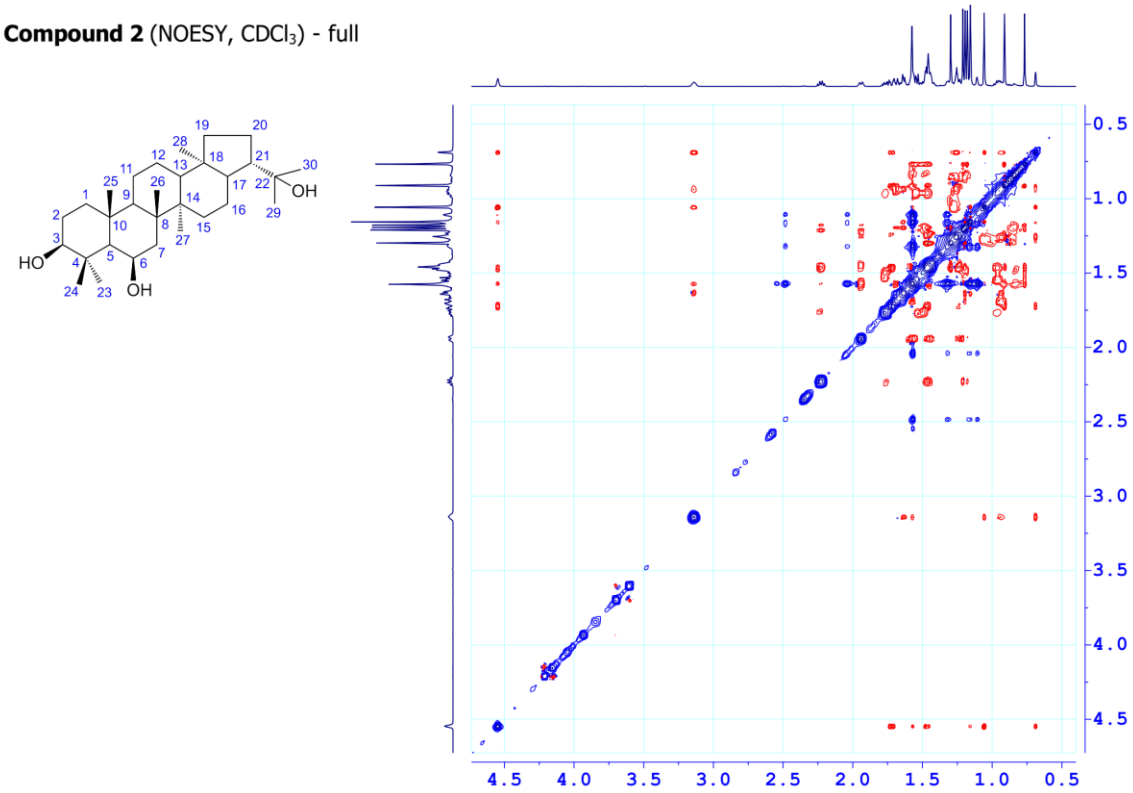


Figure S31: Full NOESY of compound **2** (21α-hopane-3β,6β,22-triol)

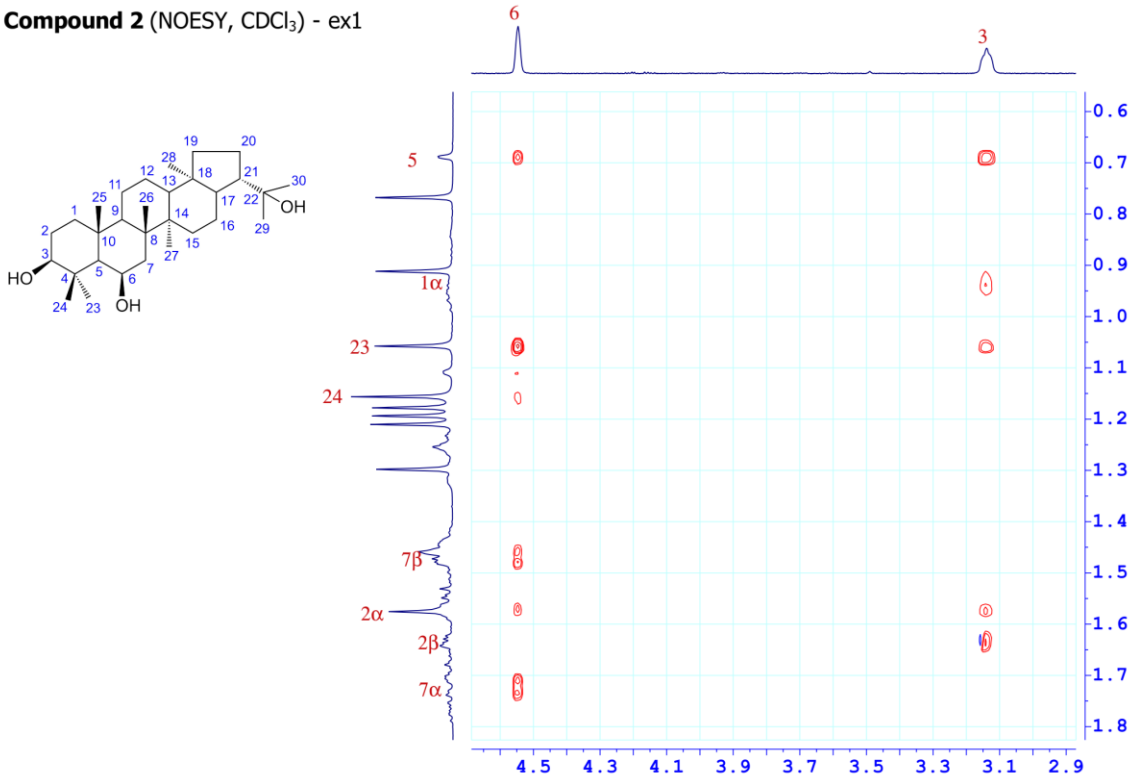


Figure S32: Extended NOESY of compound **2** (21α-hopane-3β,6β,22-triol)

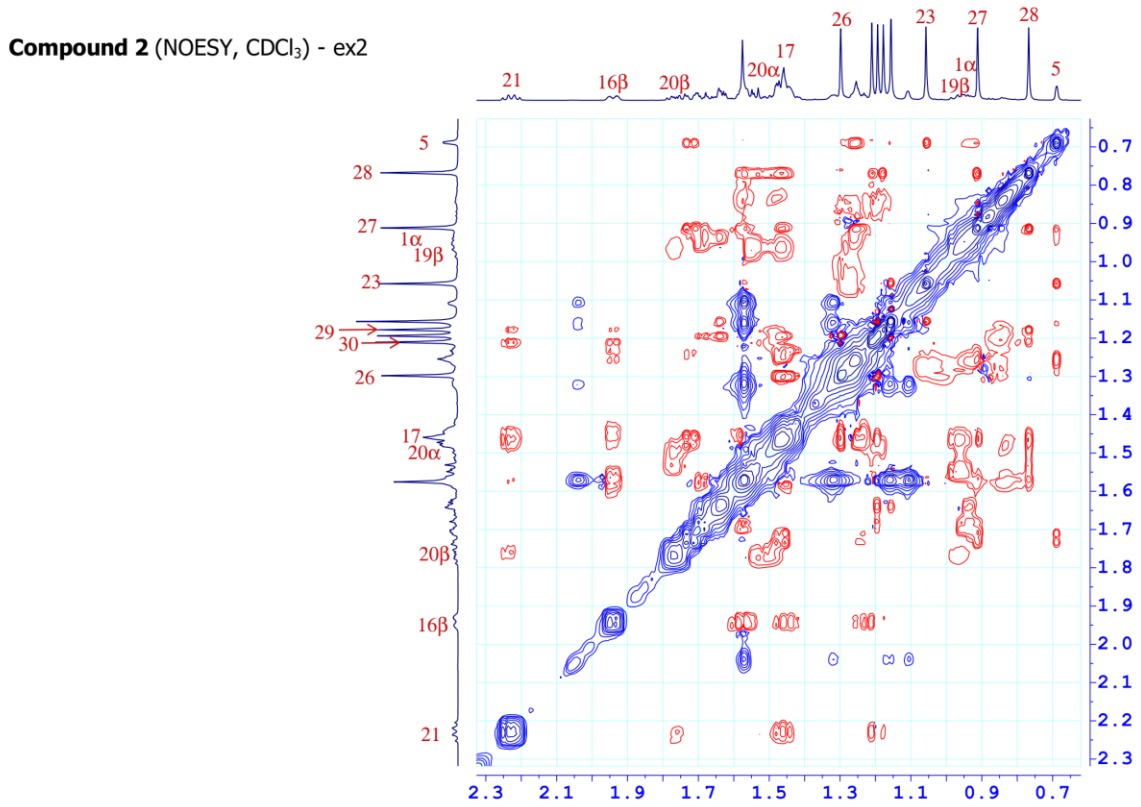
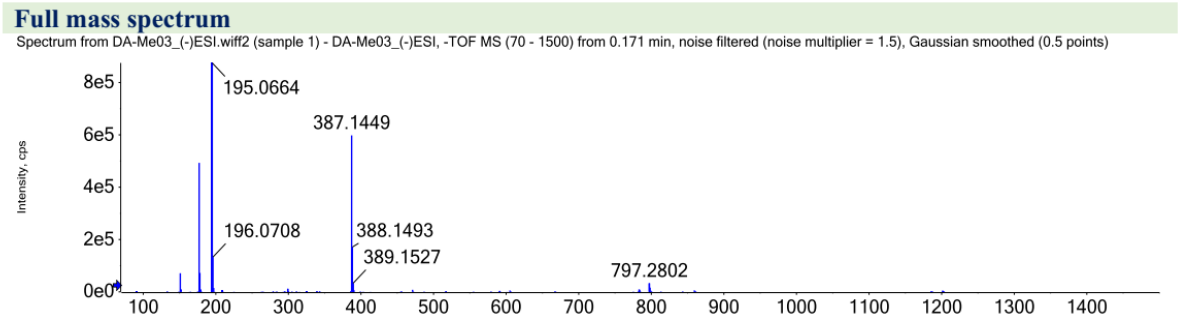


Figure S33: Extended NOESY of compound **2** (21α-hopane-3β,6β,22-triol)



Formula (M)	Ion formula	*m/z*	Calcd *m/z*	Diff (ppm)
$C_{21}H_{24}O_7$	$C_{21}H_{23}O_7^-$	387.1449	387.1449	0

Figure S34. (–)HRESI-MS of compound **3** (2′-*O*-Methylnordivaricatic acid)

Figure S35: FT-IR of compound **3** (2′-*O*-Methylnordivaricatic acid)

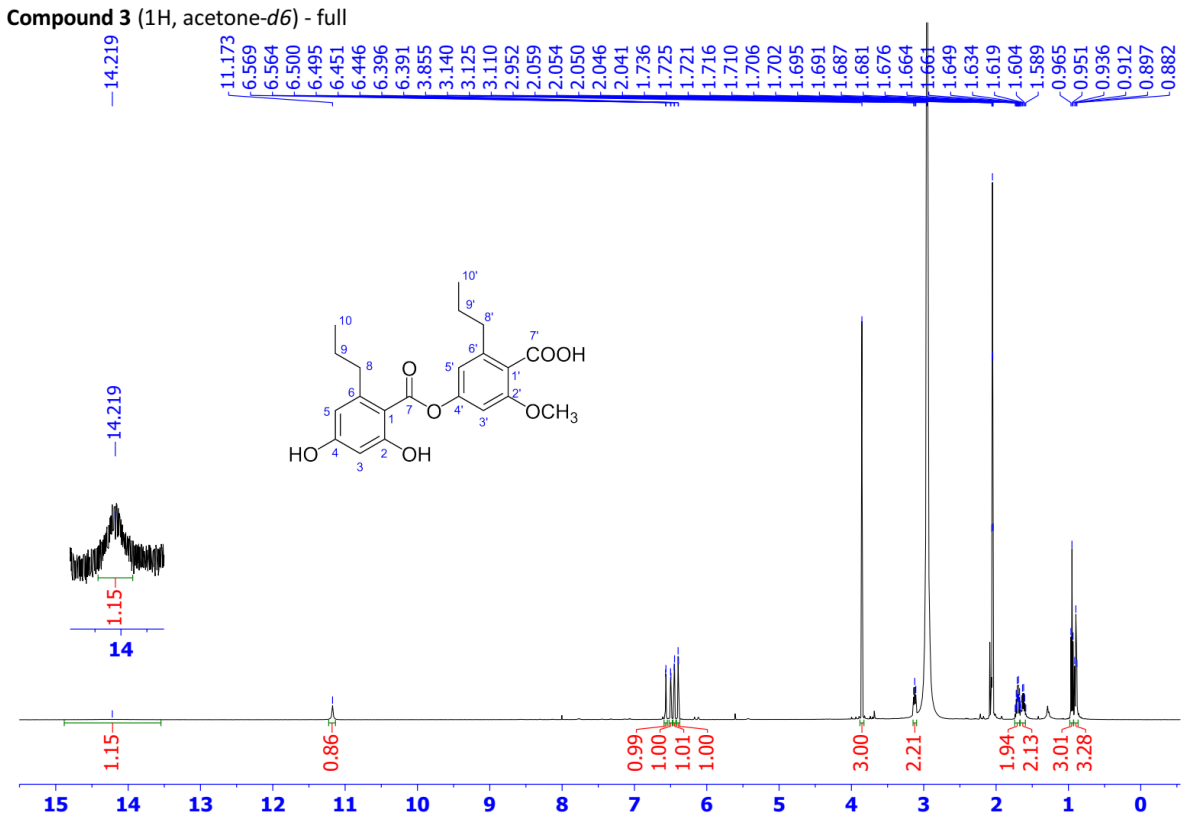


Figure S36: Full ^{1}H-NMR of compound **3** (2′-*O*-Methylnordivaricatic acid)

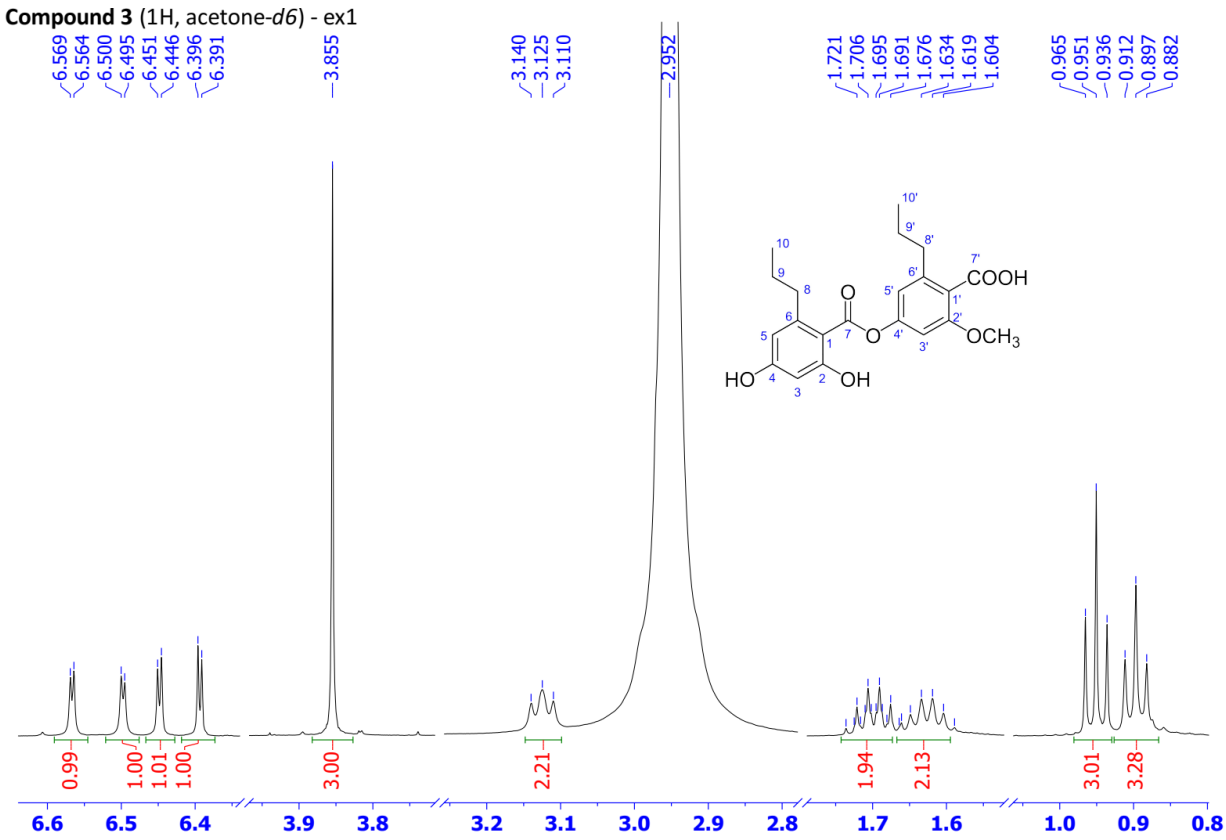


Figure S37: Extended [1]H-NMR of compound **3** (2′-*O*-Methylnordivaricatic acid)

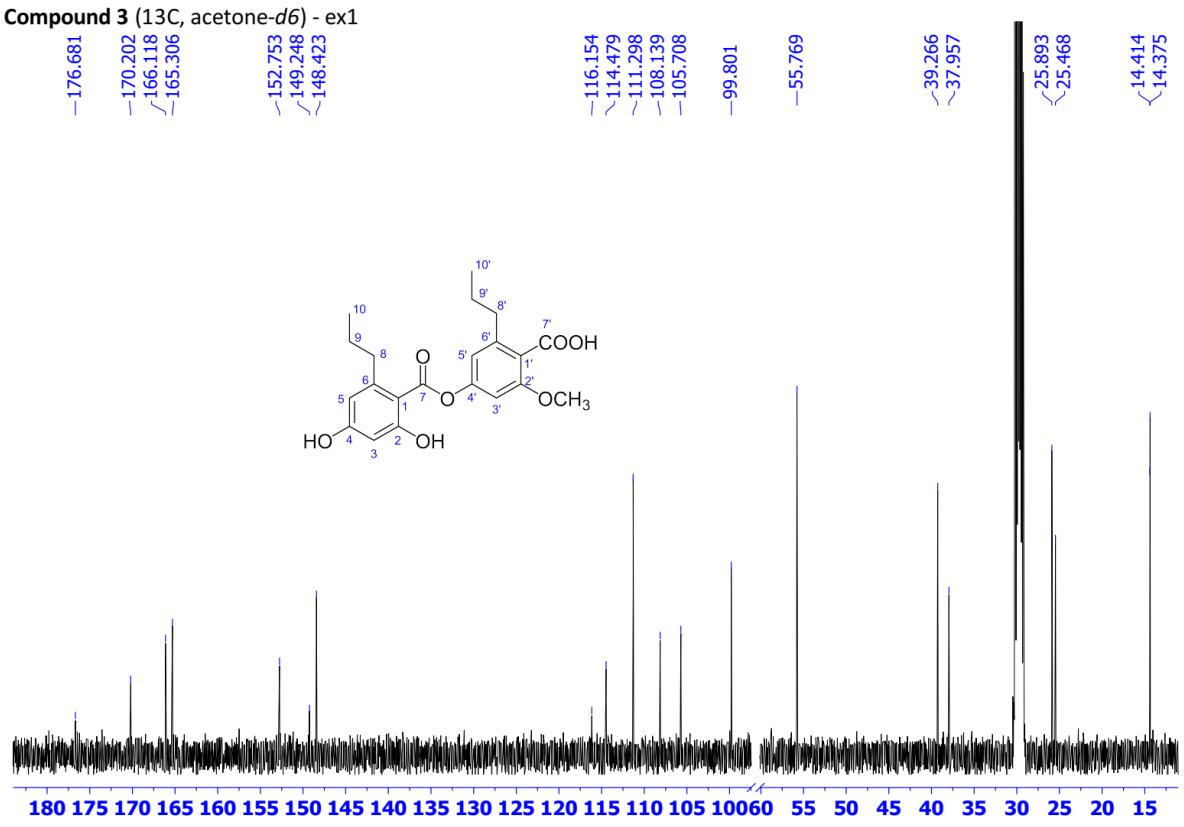


Figure S38: Full [13]C-NMR of compound **3** (2′-*O*-Methylnordivaricatic acid)

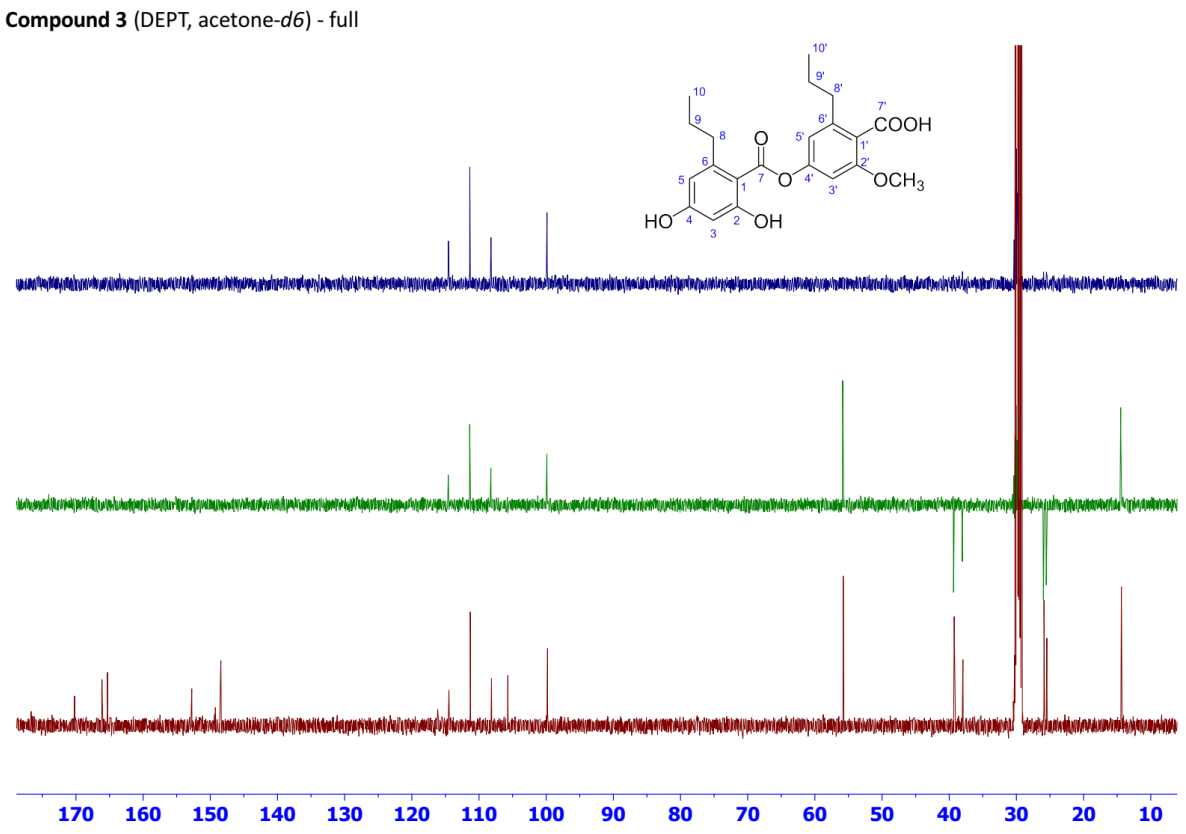


Figure S39: Full DEPT of compound **3** (2′-*O*-Methylnordivaricatic acid)

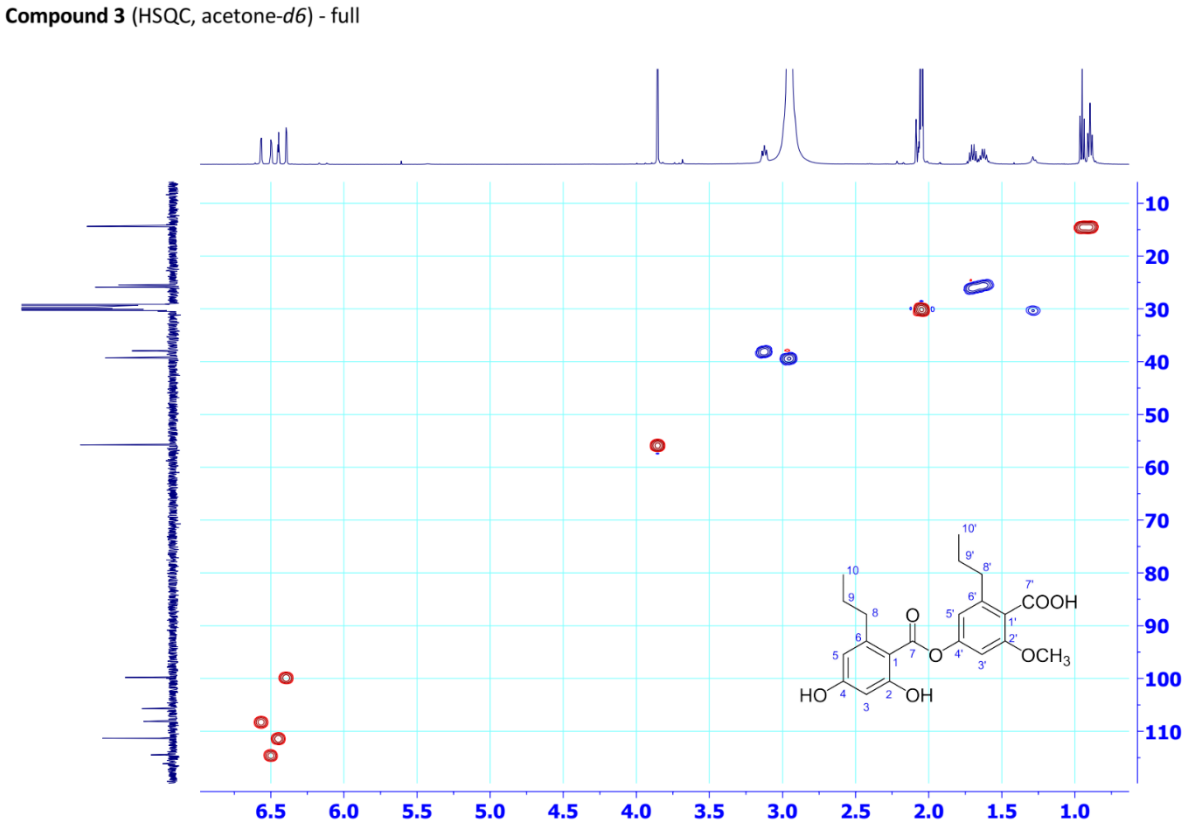


Figure S40: Full HSQC of compound **3** (2′-*O*-Methylnordivaricatic acid)

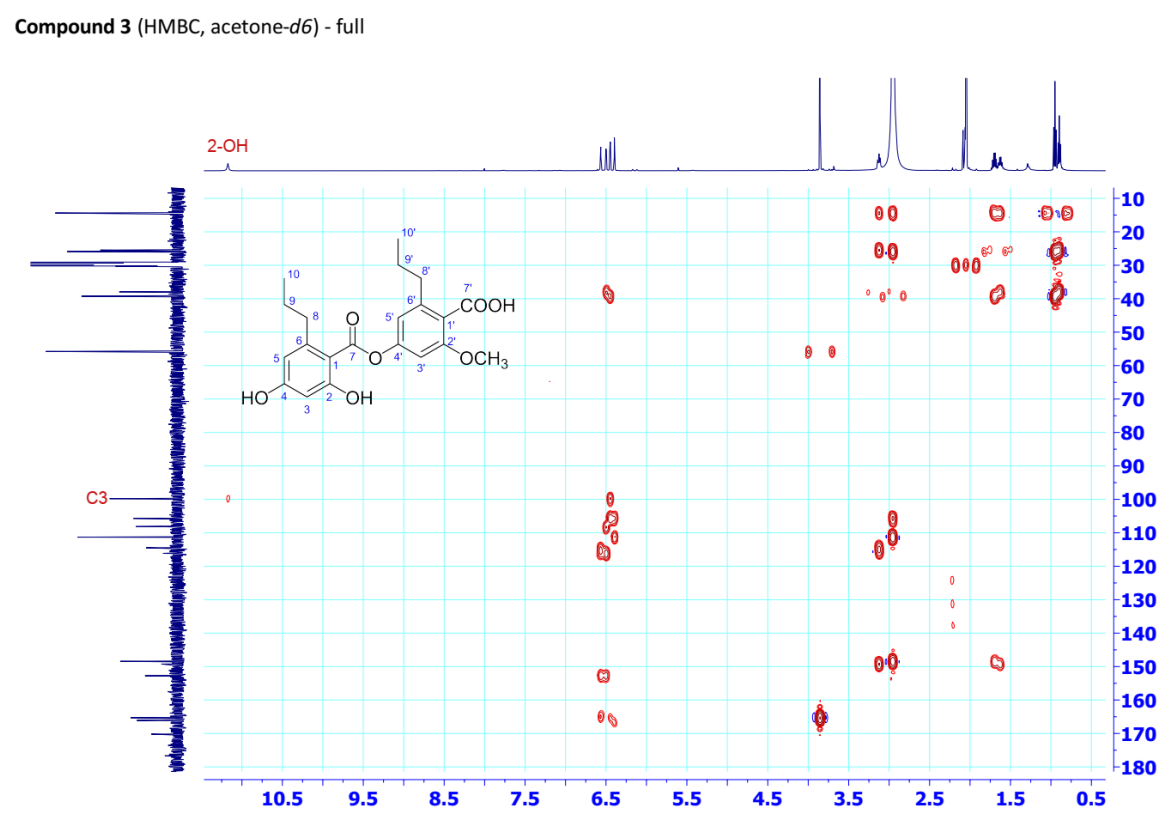


Figure S41: Full HMBC of compound **3** (2′-*O*-Methylnordivaricatic acid)

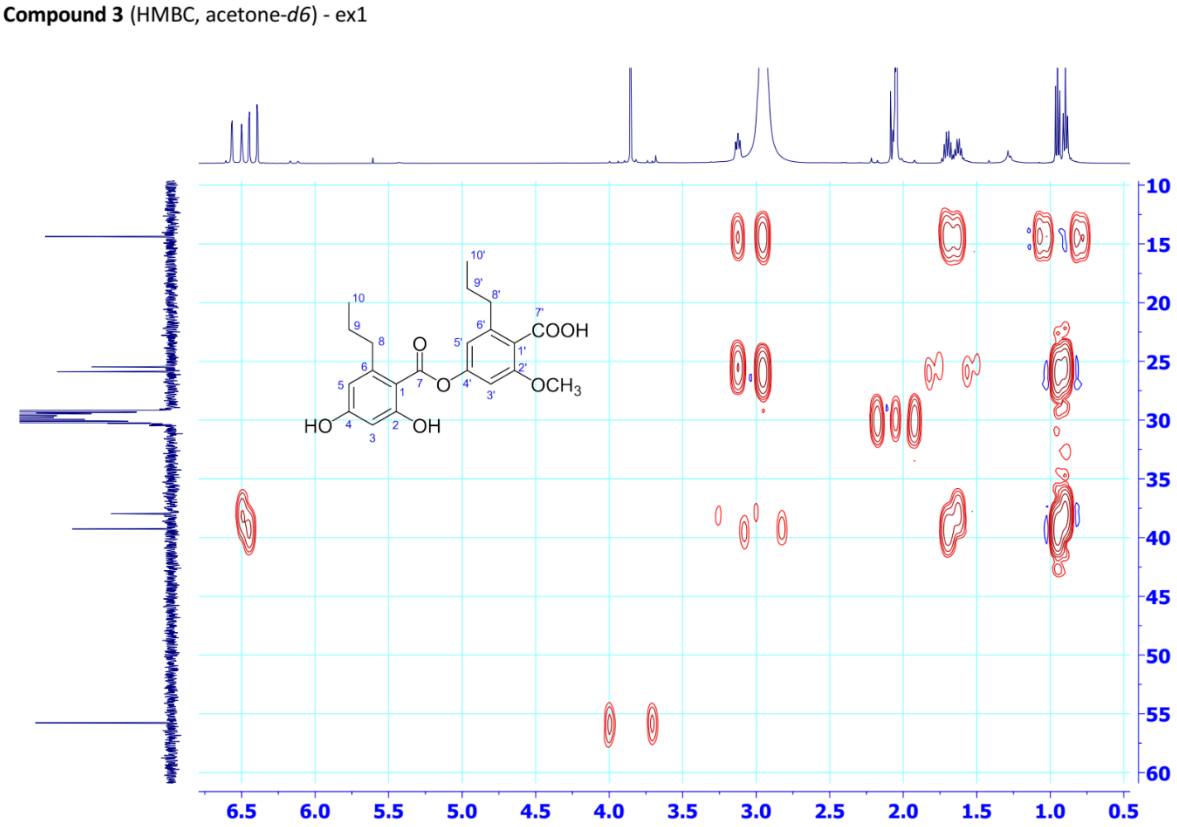


Figure S42: Extended HMBC of compound **3** (2′-*O*-Methylnordivaricatic acid)

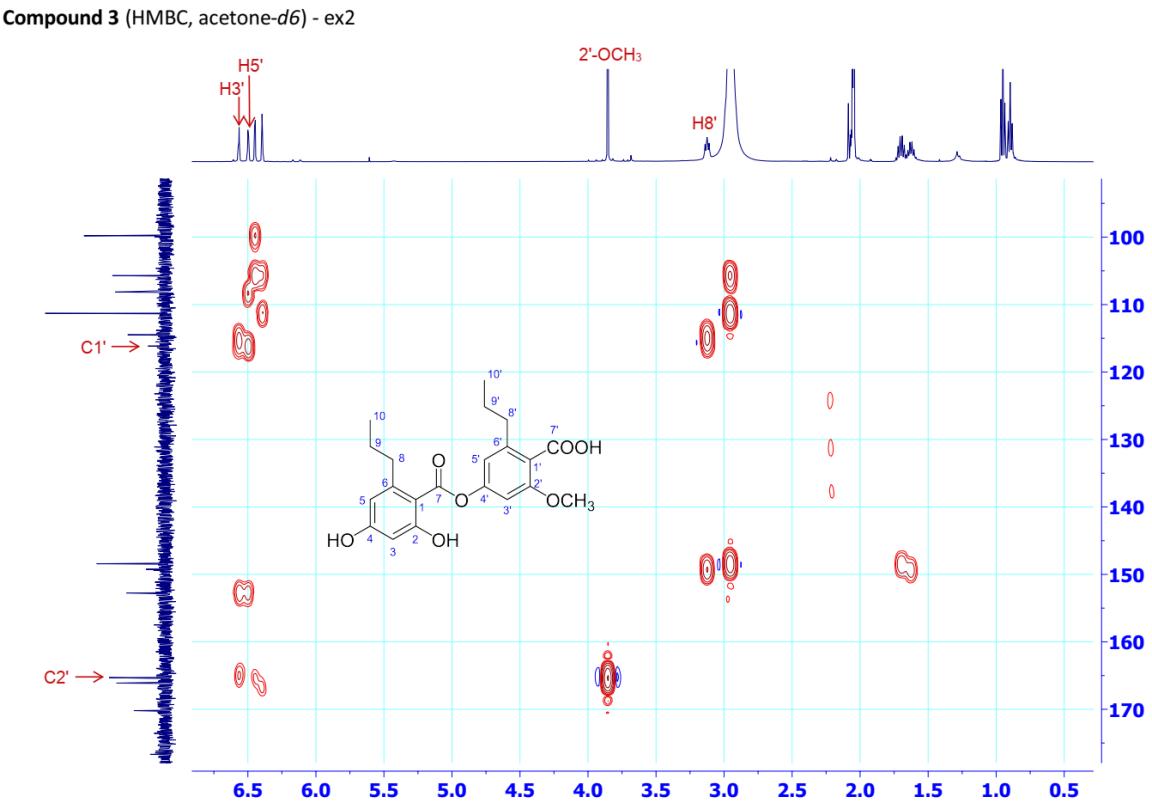


Figure S43: Extended HMBC of compound **3** (2′-*O*-Methylnordivaricatic acid)