Supporting Information

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Identification and Quantification, by NMR and LC-MS, of Sterols Isolated from the Marine Sponge *Aplysina aerophoba*

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Table S1. ^{13}C-NMR chemical shifts of the major sterol, aplysterol (**1**), and its acetate (**2**) (75 MHz, $CDCl_3$). The ^{13}C-NMR spectral data are consistent with both the values reported in the literature [13] and the DEPT spectral data.

Carbon No.	**Aplysterol (1) δ (ppm)**	**Aplysteril Acetate (2) δ (ppm)**
1 (CH_2)	37.2	37.0
2 (CH_2)	31.7	27.7
3 (CH)	71.8	74.0
4 (CH_2)	42.3	38.1
5 (C)	140.7	139.6
6 (CH)	121.7	122.6
7 (CH_2)	31.9	31.9
8 (CH)	32.0	31.9
9 (CH)	50.1	50.0
10 (C)	36.5	37.0
11 (CH_2)	21.1	21.0
12 (CH_2)	39.8	39.8
13 (C)	39.7	39.7
14 (CH)	56.8	56.7
15 (CH_2)	24.3	24.3
16 (CH_2)	28.2	28.2
17 (CH)	56.1	56.1
18 (CH_3)	11.8	11.8
19 (CH_3)	19.4	19.3
20 (CH)	35.9	35.8
21 (CH_3)	18.8	18.7
22 (CH_2)	33.9	33.8
23 (CH_2)	29.0	29.0
24 (CH)	39.8	39.8
25 (CH)	37.5	38.1
26 (CH_2)	25.8	25.7
27 (CH_3)	15.9	15.9
28 (CH_3)	16.5	16.5
29 (CH_3)	12.2	12.0
CH_3**C**OO-		170.5
CH_3COO-		21.4

Table S2. Potential molecular structures from the parent ions obtained.

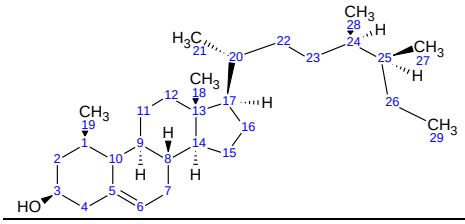
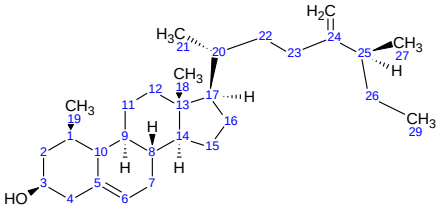
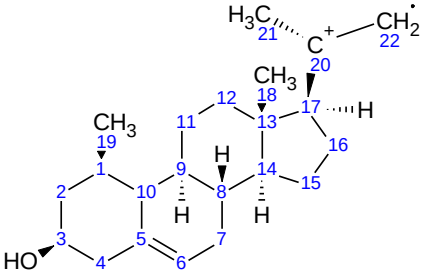
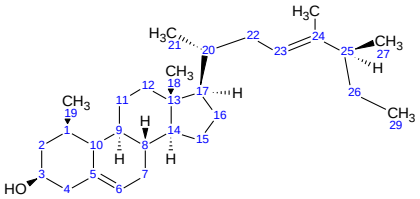
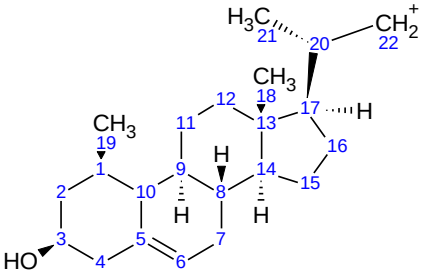
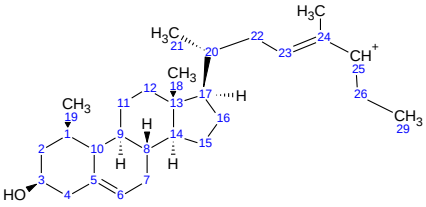
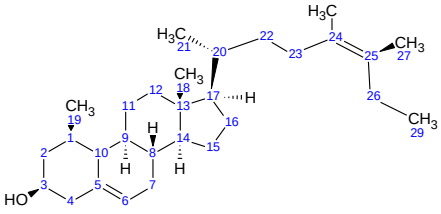
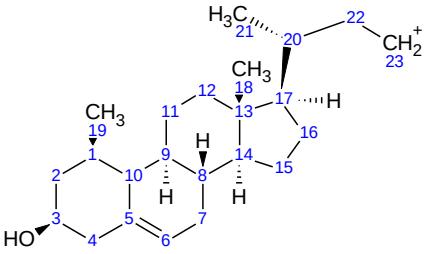
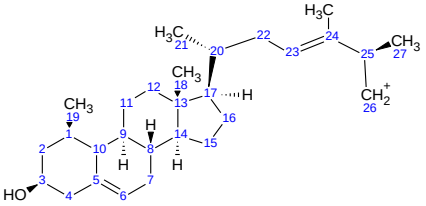
Aplysterol			
Parent ions		Daugther ions	
	414.71		
Didehydroaplysterol			
Parent ions		Daugther ions	
	412.69		314.25
	412.69		315.25
			397.69
	412.69		329.54
			397.69

Table S3. Gradient used in the chromatographic separations

Time (min)	% A	% B
0.0	50	50
5.0	0	100
7.5	0	100
11.0	50	50
15.0	50	50

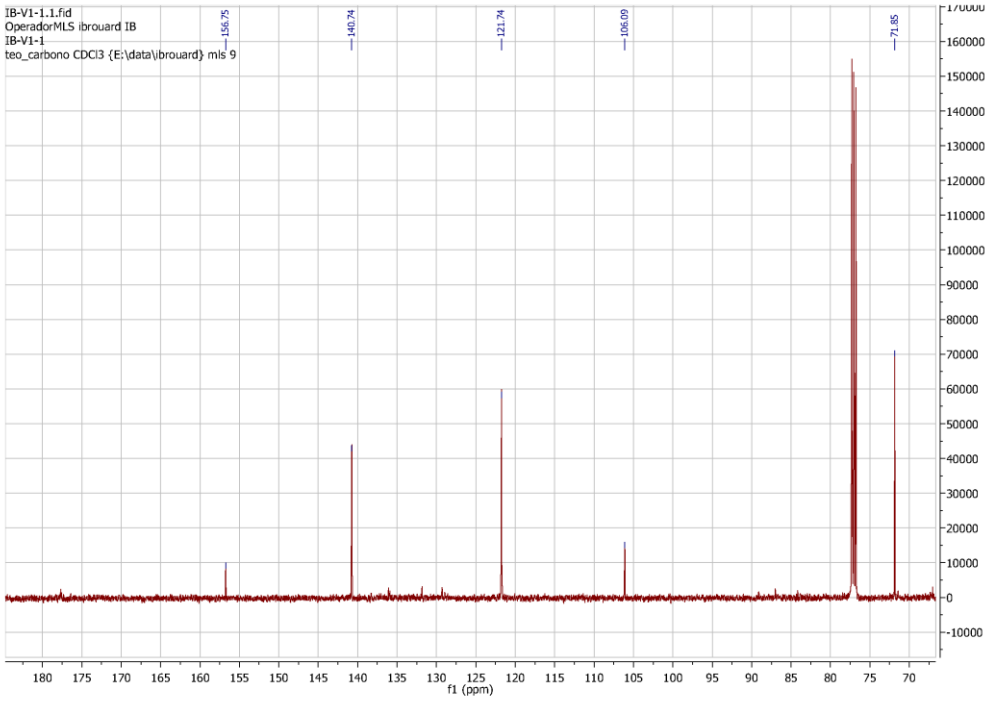


Figure S1. Signals produced by the olefinic carbons of the aplysterol mixture in the ^{13}C-NMR spectrum (125 MHz, $CDCl_3$). The relative intensities of the four equivalent quaternary carbons shown in Table 1 give the quantitative percentage for the mixture described in Figure 1.

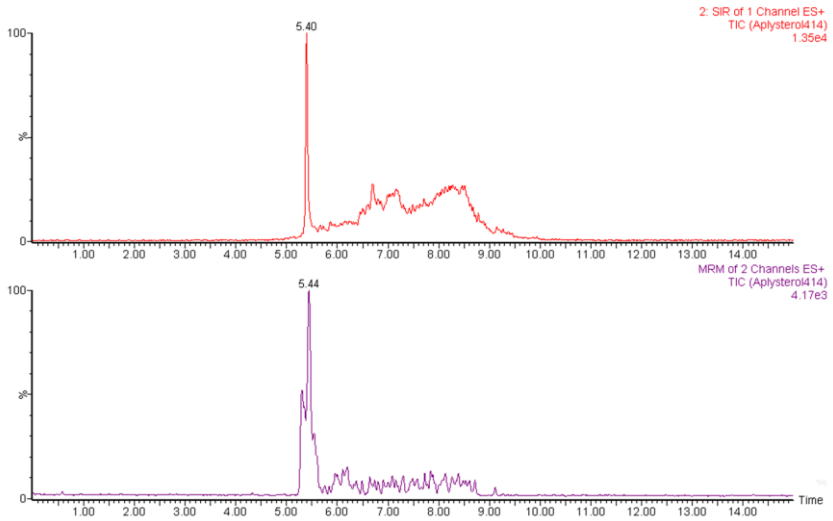


Figure S2. Aplysterol chromatogram in SIR and MR modes (UHPLC-MS).

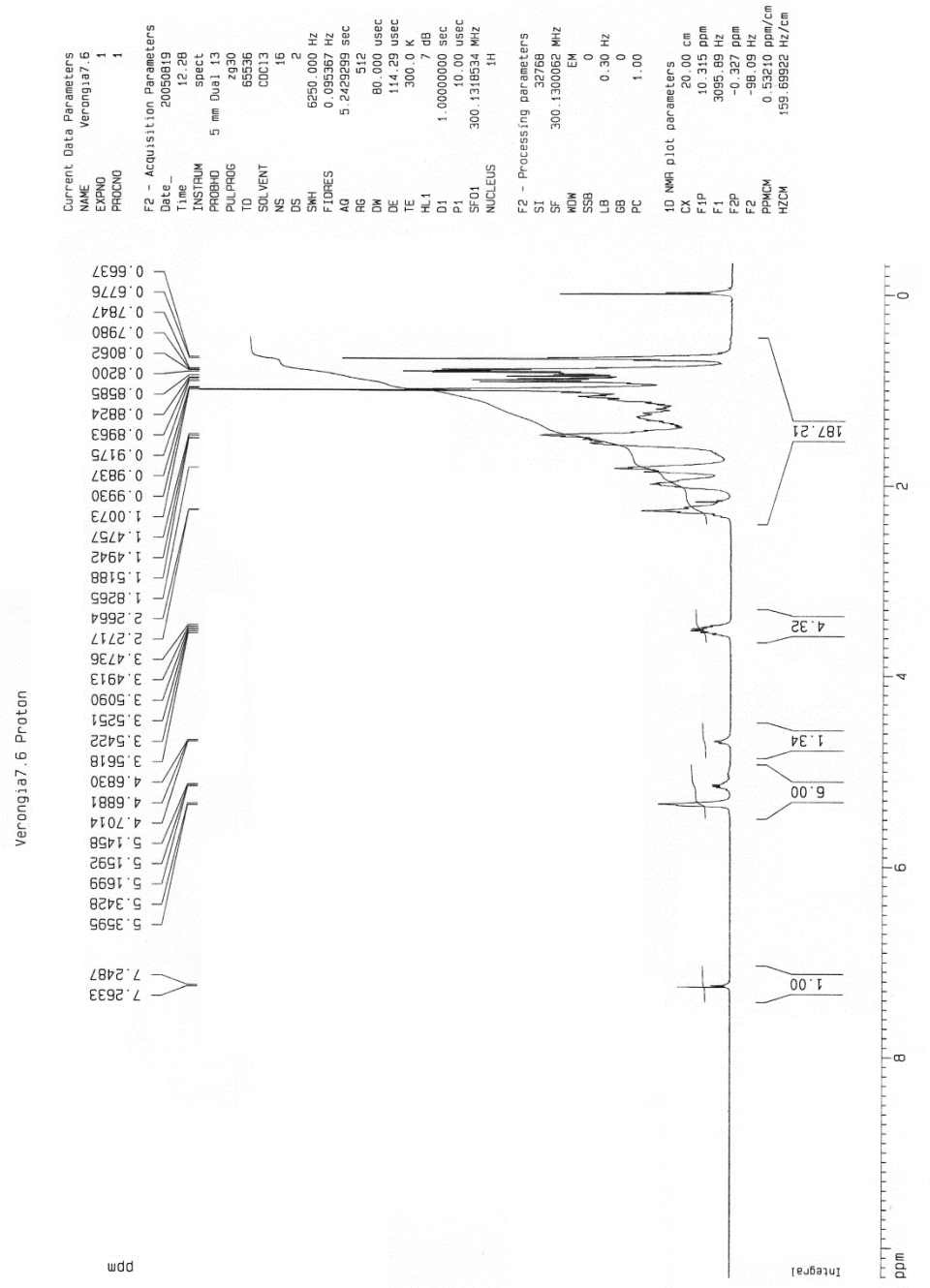


Figure S3. ^{1}H-NMR spectrum of the mixture of aplysterols (300 MHz, $CDCl_3$).

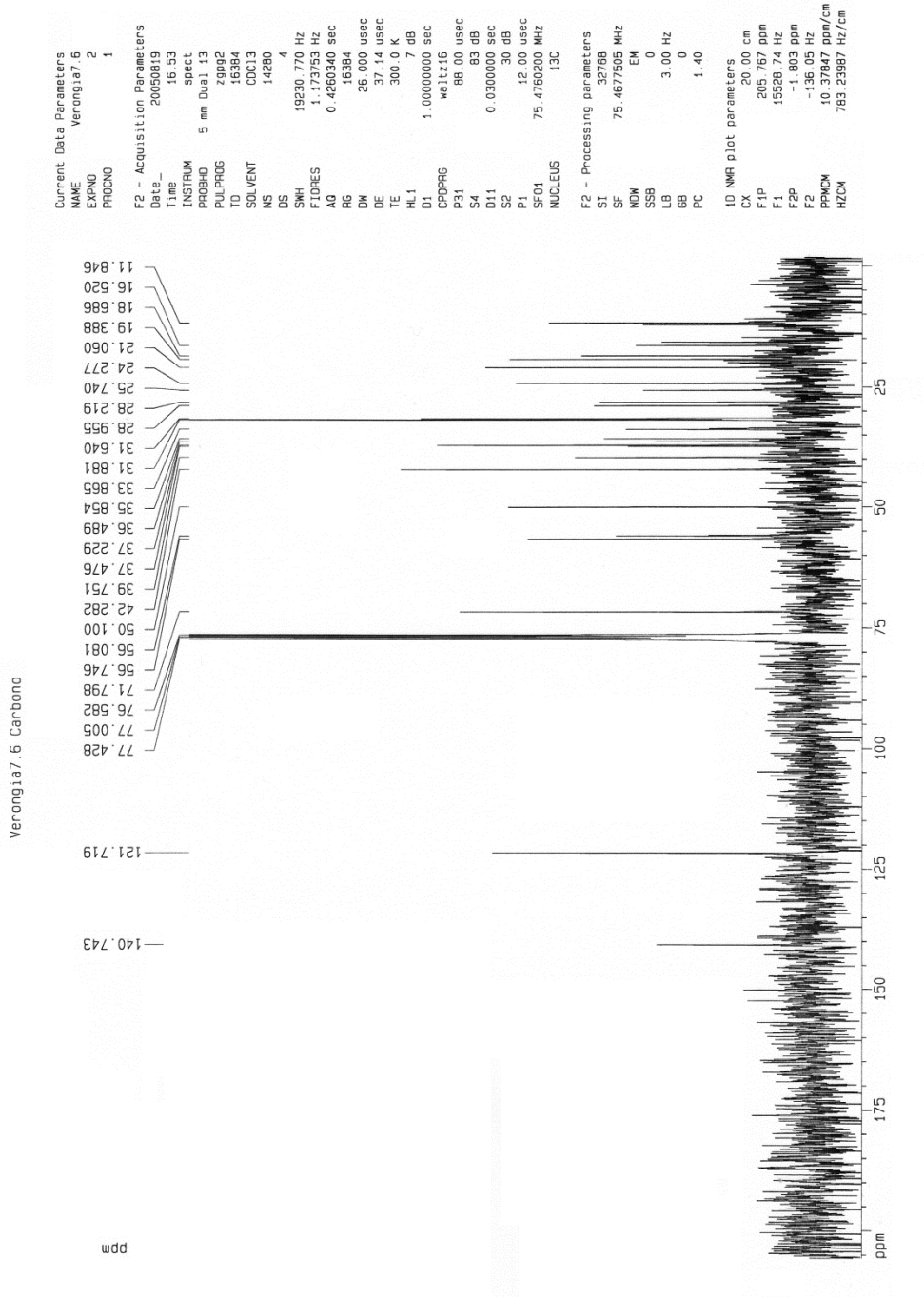


Figure S4. ^{13}C-NMR spectrum of the mixture of aplysterols (75 MHz, $CDCl_3$).

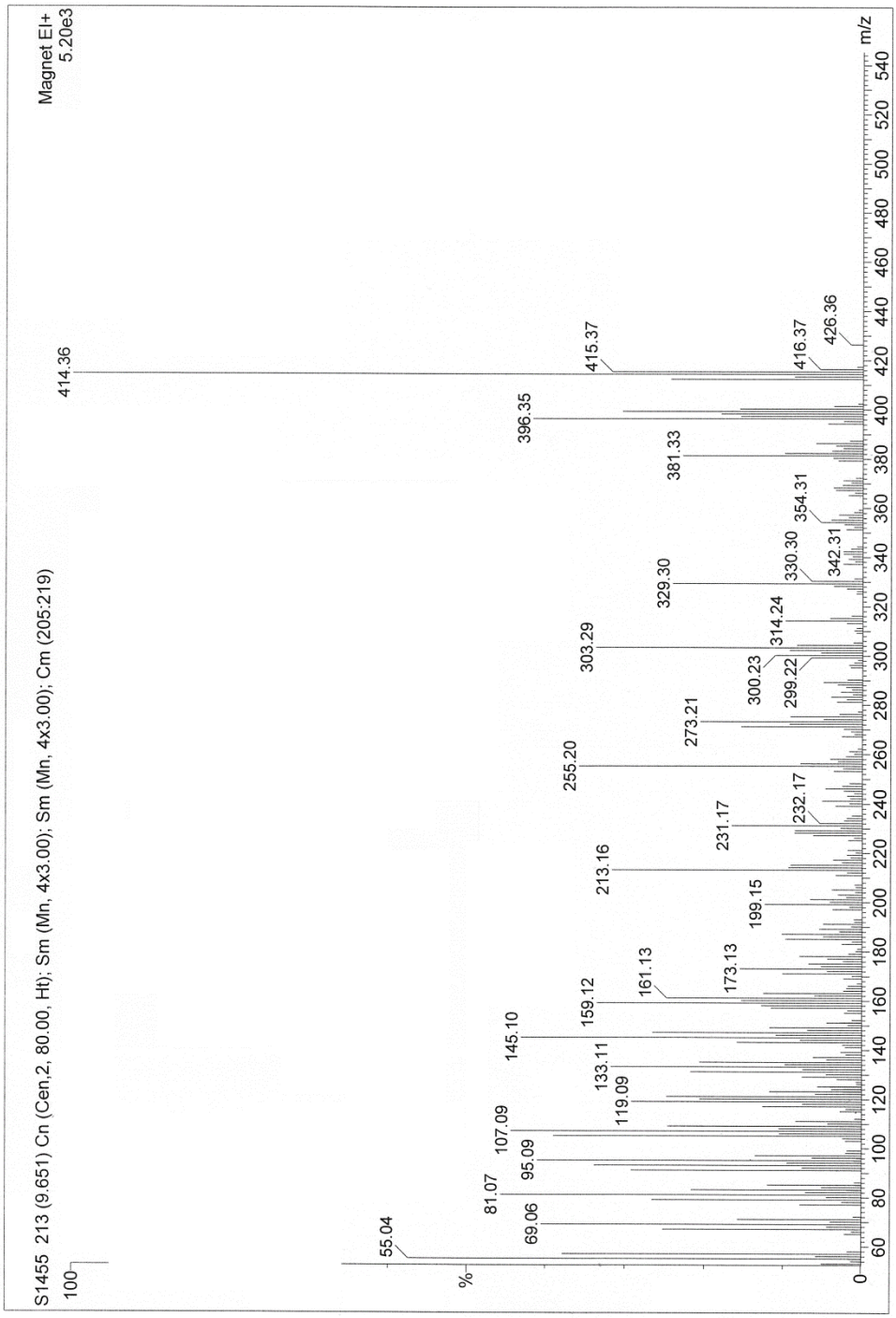
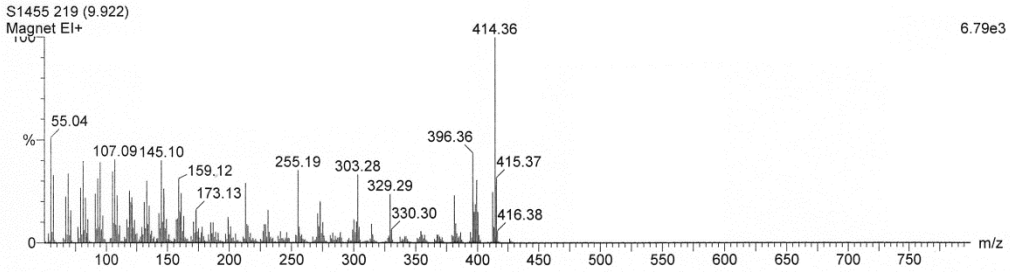


Figure S5. Mass spectrum of the mixture of aplysterols.

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2133 mass(es) processed - displaying only valid results
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Isotope matching not enabled

Monoisotopic Mass, Odd and Even Electron Ions
26784 formula(e) evaluated with 235 results within limits (up to 50 closest results for each mass)



Minimum:	5.00				-1.5			
Maximum:	100.00		200.0	10.0	50.0			
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula		
416.3680	5.35	416.3654	2.6	6.2	5.0	C28	H48	O2
416.3641	5.35	416.3654	-1.3	-3.2	5.0	C28	H48	O2
415.4164	5.82	415.4151	1.3	3.1	-0.5	C26	H55	O3
415.4125	7.10	415.4151	-2.6	-6.3	-0.5	C26	H55	O3
415.3972	18.82	415.3940	3.2	7.7	4.5	C29	H51	O
415.3933	23.89	415.3940	-0.7	-1.7	4.5	C29	H51	O
415.3818	27.82	415.3787	3.1	7.4	0.5	C25	H51	O4
415.3781	28.29	415.3787	-0.6	-1.5	0.5	C25	H51	O4
415.3589	30.87	415.3576	1.3	3.1	5.5	C28	H47	O2
415.3550	28.82	415.3576	-2.6	-6.3	5.5	C28	H47	O2
415.3435	22.27	415.3424	1.1	2.8	1.5	C24	H47	O5
415.3397	19.09	415.3424	-2.7	-6.4	1.5	C24	H47	O5
		415.3365	3.2	7.8	10.5	C31	H43	
415.3358	14.86	415.3365	-0.7	-1.6	10.5	C31	H43	
415.3243	5.66	415.3212	3.1	7.4	6.5	C27	H43	O3
414.4248	5.24	414.4226	2.2	5.4	4.0	C30	H54	
414.4210	7.45	414.4226	-1.6	-3.7	4.0	C30	H54	
414.4095	18.54	414.4073	2.2	5.3	0.0	C26	H54	O3
414.4057	26.89	414.4073	-1.6	-3.9	0.0	C26	H54	O3
414.3866	75.39	414.3862	0.4	1.0	5.0	C29	H50	O
414.3828	80.56	414.3862	-3.4					
414.3713	95.39	414.3709	0.4					
414.3675	98.11	414.3709	-3.4					
414.3522	95.30	414.3498	2.4					
414.3484	89.82	414.3498	-1.4					
414.3369	67.32	414.3345	2.4					
414.3331	55.94	414.3345	-1.4					
414.3293	43.64	414.3287	0.6					
414.3254	30.24	414.3287	-3.3					
414.3140	6.82	414.3134	0.6					
413.3747	5.27	413.3783	-3.6					
413.3670	7.11	413.3631	3.9					
413.3632	7.17	413.3631	0.1					
413.3594	7.32	413.3631	-3.7					
413.3441	7.08	413.3420	2.1					
413.3403	7.78	413.3420	-1.7					
413.3289	6.85	413.3267	2.2					
413.3251	7.20	413.3267	-1.6					
413.3213	6.14	413.3208	0.5					
412.3880	6.55	412.3916	-3.6					
412.3728	17.69	412.3705	2.3					
412.3690	18.94	412.3705	-1.5	-3.7	6.0	C29	H48	O
412.3576	24.71	412.3553	2.3	5.7	2.0	C25	H48	O4

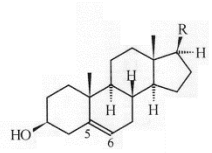


Figure S6. High resolution mass spectrum of the mixture of aplysterols.

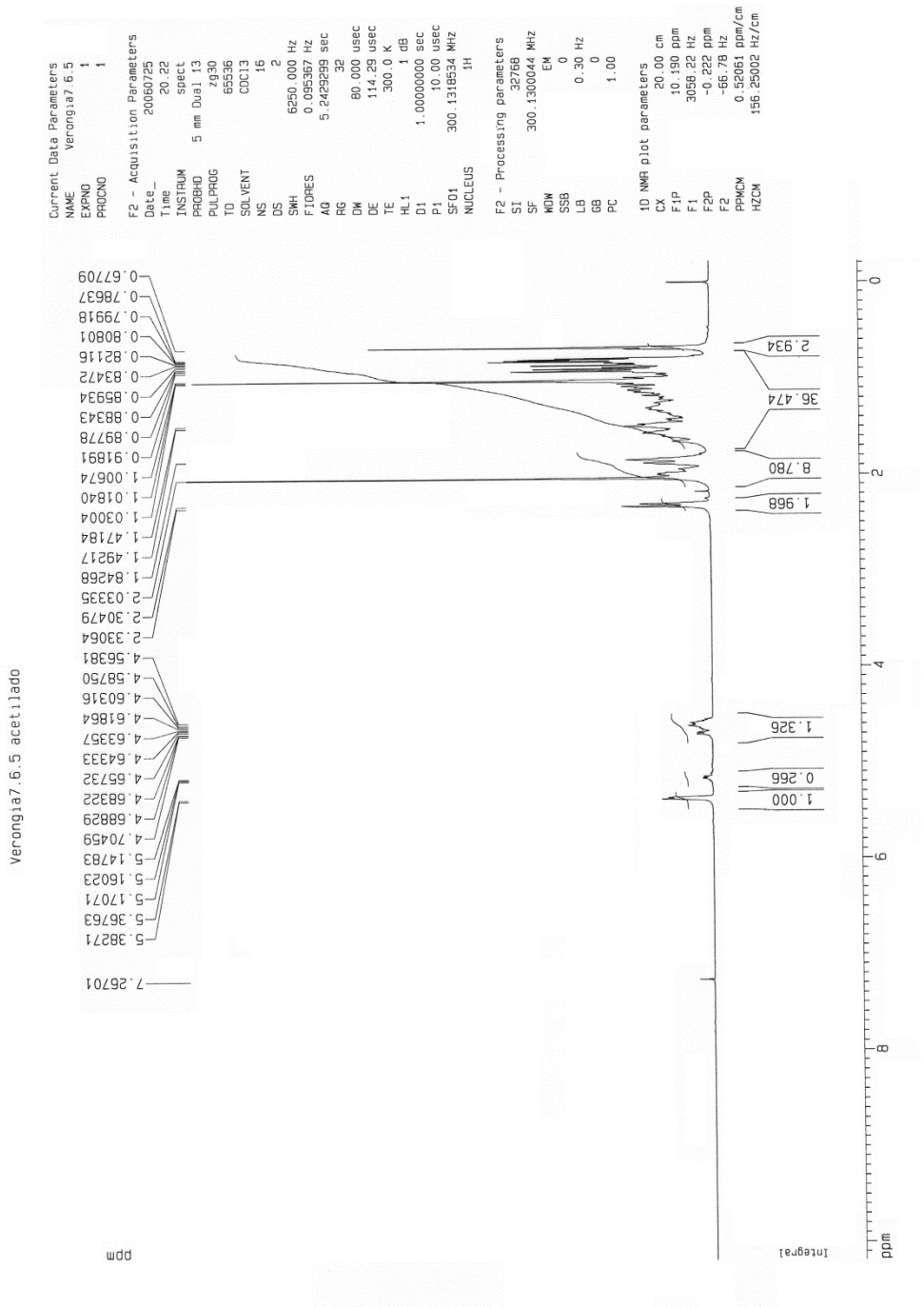


Figure S7. ^{1}H-NMR spectrum of the mixture of aplysterol acetates (300 MHz, $CDCl_3$).

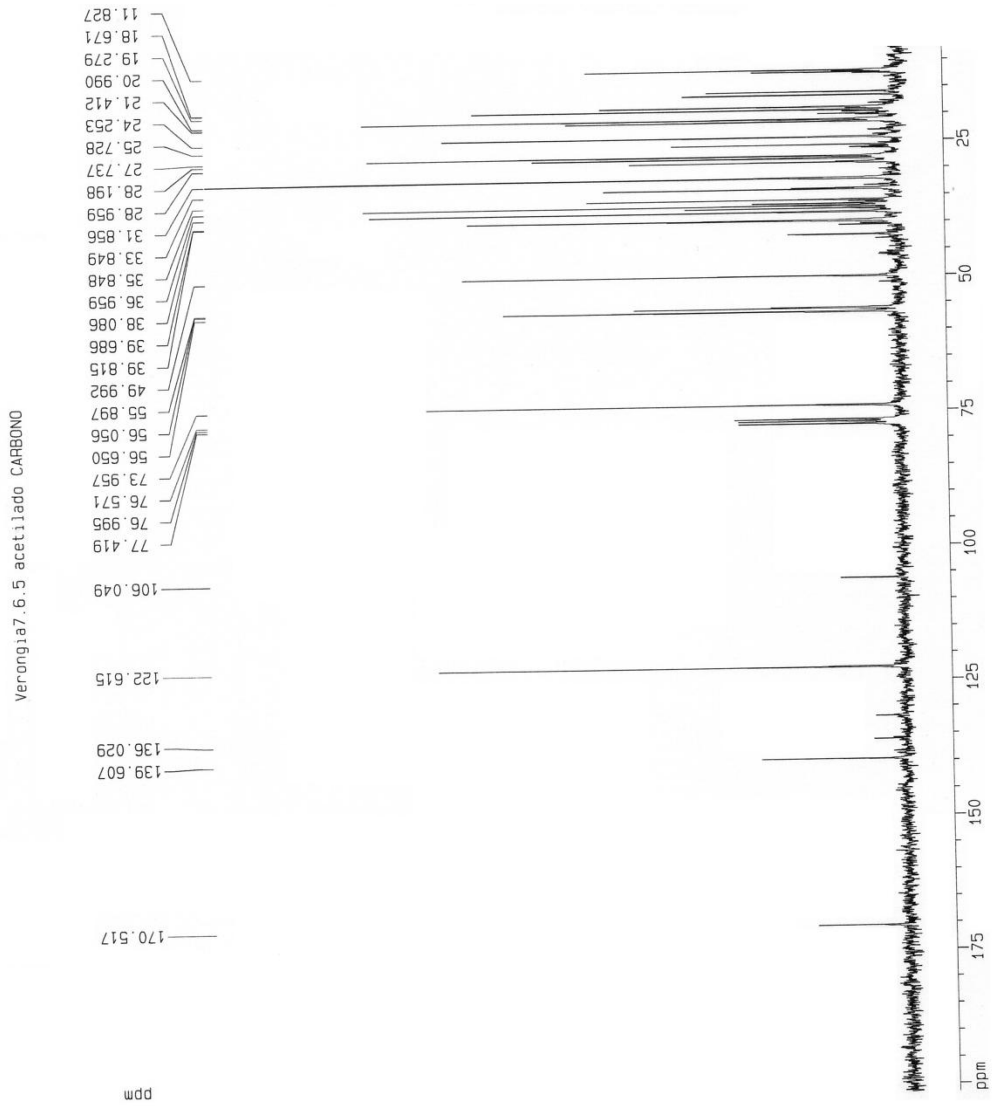
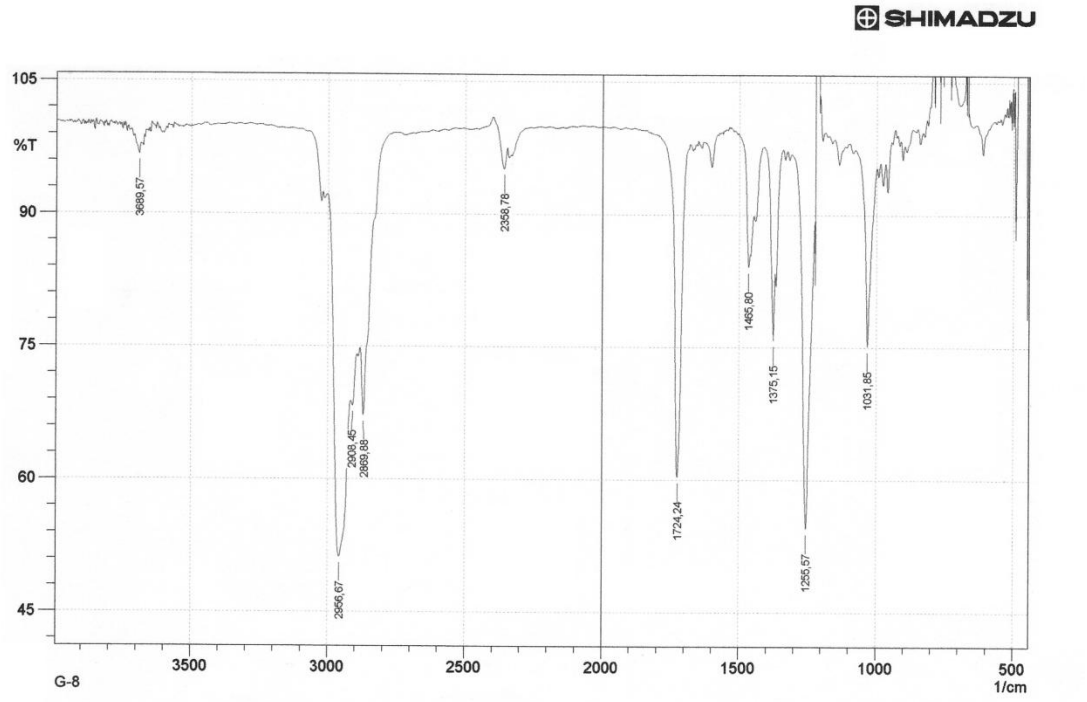


Figure S8. ^{13}C-NMR spectrum of the mixture of aplysterol acetates (75 MHz, $CDCl_3$).



	Peak	Height	Corr. Height	Base (H)	Base (L)	Area	Corr. Area	
1	1031,85	24,85	21,05	1076,21	999,06	3,66	2,41	
2	1255,57	45,49	36,63	1307,65	1226,64	8,63	5,8	
3	1375,15	24,19	9,63	1409,87	1369,37	2,1	0,48	
4	1465,8	15,91	8,76	1502,44	1448,44	1,94	0,68	
5	1724,24	39,67	38,01	1789,82	1681,81	6,39	5,64	
6	2358,78	4,84	2,97	2401,21	2349,14	0,45	0,27	
7	2869,88	32,8	9,93	2881,45	2761,87	6,52	0,95	
8	2908,45	31,7	2,04	2914,24	2894,95	2,91	0,12	
9	2956,67	48,78	28,51	3002,96	2916,17	16,67	8,01	
10	3689,57	3,33	1,39	3708,86	3683,78	0,26	0,08	

Figure S9. IR spectrum of the mixture of aplysterol acetates.

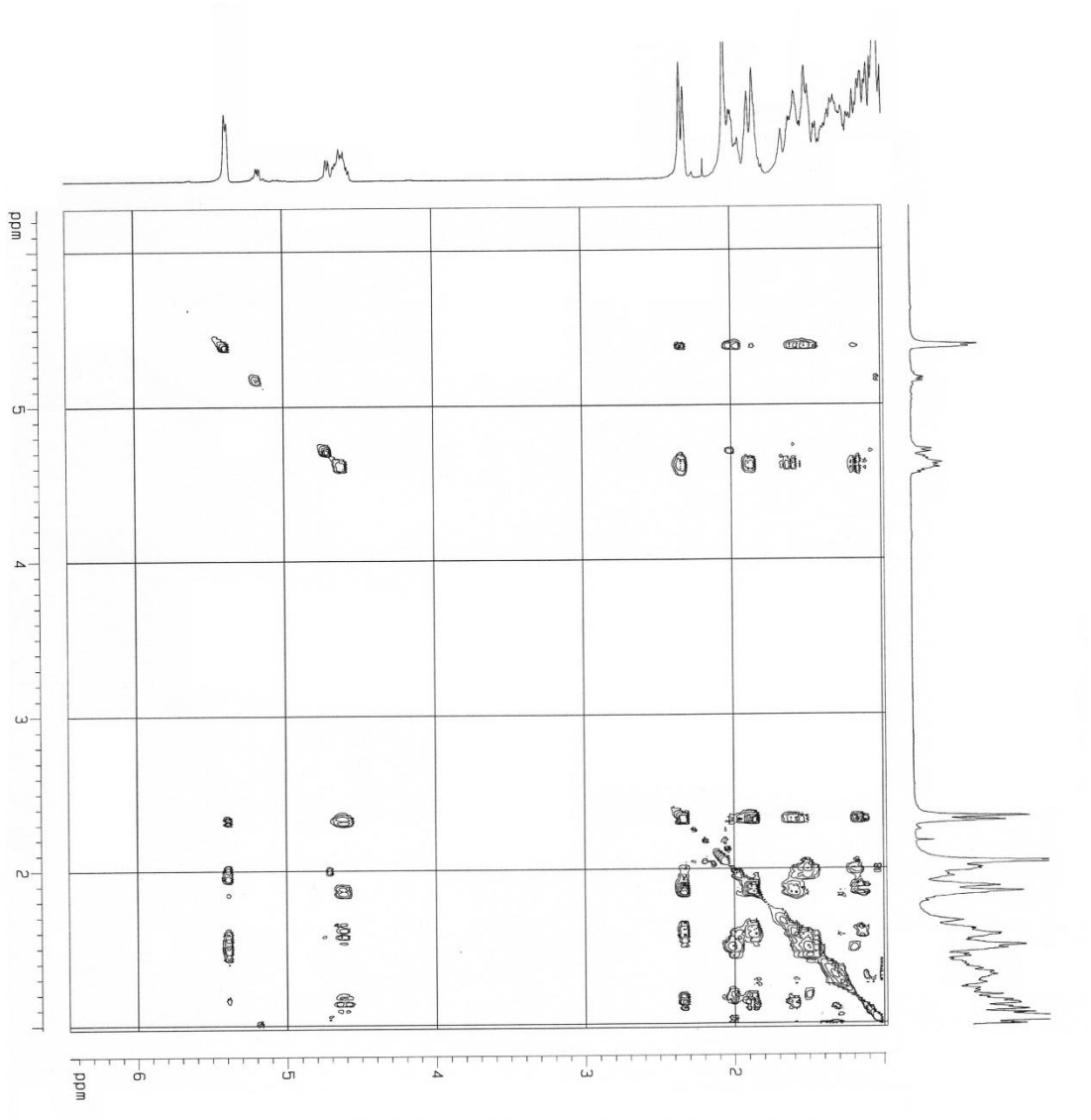


Figure S10. TOCSY spectrum of the mixture of aplysterol acetates (300 MHz, $CDCl_3$).