

SUPPLEMENTARY MATERIAL

Microbial transformation of isocoronarin D by *Cunninghamella echinulata* NRRL 1386

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Abstract

The diterpene isocoronarin D (**1**) is a bioactive major constituent of labdane diterpene from the aerial parts of *Curcuma comosa* Roxb. (Zingiberaceae), the Thai medicinal plant. Microbial transformation of **1** was performed by the fungus *Cunninghamella echinulata* NRRL 1386 to yield three new metabolites, 3 β -hydroxyisocoronarin D (**2**), 6 α -hydroxyisocoronarin D (**3**) and 3 β ,7 α -dihydroxyisocoronarin D (**4**). The structures of the new compounds were elucidated by spectroscopic techniques.

Key words: isocoronarin D; *Curcuma comosa*; *Cunninghamella echinulata*; microbial transformation

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Table S1 ^1H NMR (400 MHz) data of compounds **2–3** (CDCl_3)^a and compound **4** ($\text{CDCl}_3 + 6 \text{CD}_3\text{OD}$)^b

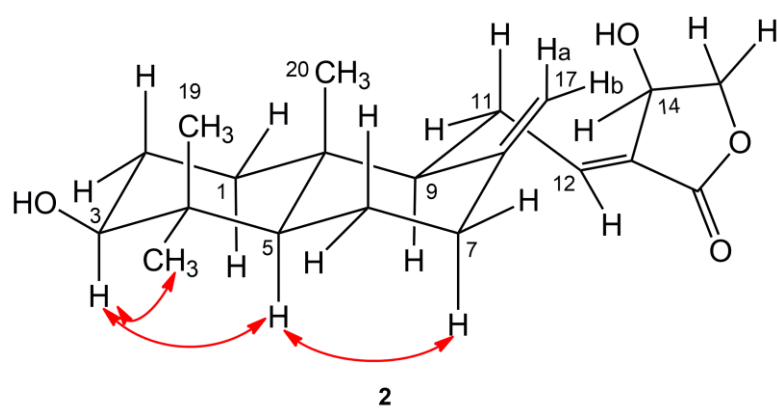
position	2	3	4
1 α	1.20 (dt, 12.6, 5.2)	1.06 ^c	1.22 (dt, 13.2, 4.8)
1 β	1.72 ^c	1.63 (br d, 12.2)	1.65 ^c
2 α	1.60 (ddd, 16.7, 12.6, 4.1)	1.47 ^c	1.58 ^c
2 β	1.75 ^c	1.52 ^c	1.65 ^c
3	3.25 (dd, 11.7, 4.1)	3 α : 1.25 ^c 3 β : 1.37 (dt, 13.4, 3.1)	3.22 (dd, 11.8, 3.9)
5	1.10 (br dd, 12.7, 1.6)	1.13 ^c	1.59 ^c
6	6 α : 1.40 (ddd, 16.8, 12.7, 3.9) 6 β : 1.73 ^c	3.83 (dt, 10.7, 4.9)	1.54 ^c 1.82 (br d, 11.8)
7	7 α : 1.98 (dt, 12.7, 4.5) 7 β : 2.41 (br d, 12.7)	7 α : 2.05 (t, 12.0) 7 β : 2.67 (dd, 12.0, 4.9)	4.30 (br s, $W_{1/2} = 4.4$)
9	1.81 (t, 6.9)	1.89 (br d, 10.5)	2.36 (br d, 9.6)
11	2.53 (br t, 6.9)	11a: 2.47 (ddd, 16.4, 10.5, 6.8) 11b: 2.57 (ddd, 16.4, 6.9, 3.5)	2.46-2.54 (m)
12	6.96 (br t, 6.7)	6.34 (br t, 6.8)	6.84 (br t, 6.3)
14	5.00 (br d, 5.3)	4.98 (br d, 5.8)	4.91 (br d, 4.9)
15 α	4.24 (br d, 10.4)	4.23 (dd, 10.4, 1.8)	4.17 (br d, 10.3)
15 β	4.43 (dd, 10.4, 6.1)	4.43 (dd, 10.4, 6.1)	4.39 (dd, 10.3, 6.1)
17a	4.56 (s)	4.62 (s)	4.66 (s)
17b	4.88 (s)	4.91 (s)	5.01 (s)
18	0.99 (s)	1.15 (s)	0.93 (s)
19	0.77 (s)	1.00 (s)	0.71 (s)
20	0.73 (s)	0.73 (s)	0.65 (s)

^{a,b} Assignments were based on ^1H – ^1H COSY, HMQC, and NOE experiments; chemical shifts are given in ppm. ^c Overlapping signal.

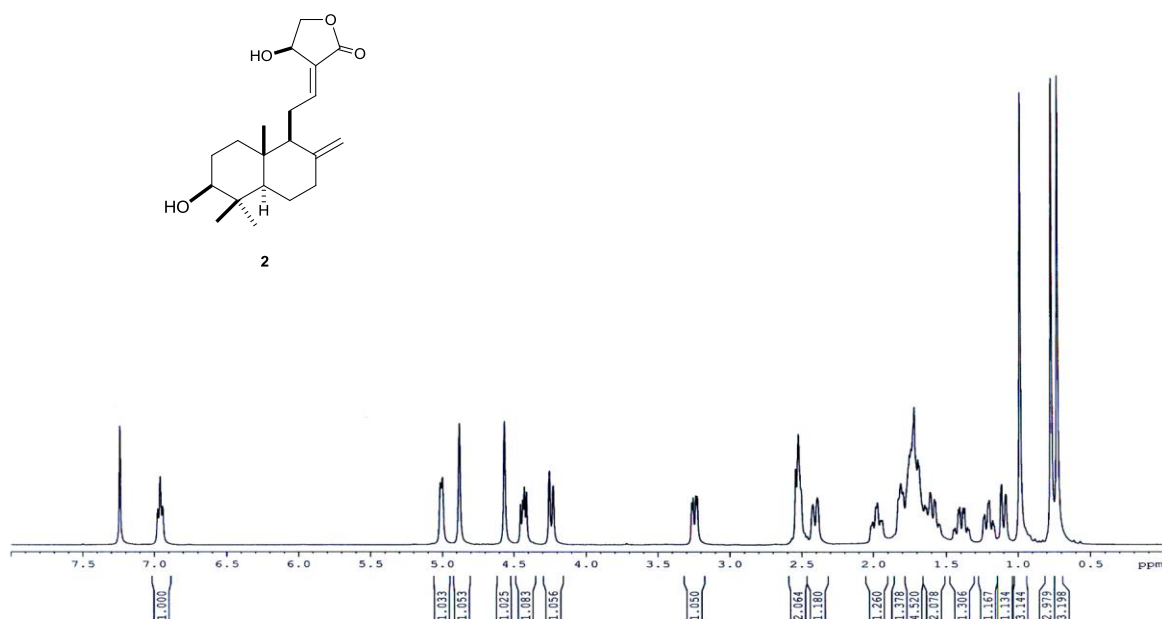
Table S2 ^{13}C NMR (100 MHz) data of compounds **2–3** (CDCl_3)^a and compound **4** (CDCl_3 + 6 CD_3OD)^b

position	2		3		4	
	δ_{C}	HMBC	δ_{C}	HMBC	δ_{C}	HMBC
1	37.2	C-2, 3, 5, 9, 10, 20	39.6	C-2, 3, 5, 10, 20	36.9	C-2, 3, 5, 9, 10, 20
2	27.7	C-3, 4	19.0	C-1, 3, 4, 10	27.4	C-1, 3, 4, 5,
3	78.6	C-1, 4, 18,19	43.6	C-4	78.3	C-4, 18, 19
4	39.1		33.9		38.5	
5	54.5	C-3, 6, 9, 18,19	60.3	C-4, 6, 7, 9, 10, 18, 19, 20	46.6	C-9, 18, 19, 20
6	23.6	C-5, 7, 8, 10	71.2	C-4, 5, 7 10	30.1	C-4, 5, 7, 10
7	37.6	C-5,6, 8, 9, 17	48.5	C-5, 6, 8,17	73.0	C-5, 9, 17
8	147.2		144.9		148.7	
9	56.0	C-5, 8, 10, 11, 12, 17, 20	55.6	C-8, 10, 11, 12, 17, 20	50.2	C-5, 10, 11, 20
10	39.2		39.2		39.2	
11	24.7	C-8, 9, 10, 12, 13	24.9	C-7, 8, 9, 10, 12, 13	24.1	C-8, 9, 10, 12, 13
12	149.1	C-9,11,13,14,16	149.1	C-9, 14, 16	148.6	
13	127.8		127.9		128.2	
14	66.2	C-12,13,16	66.1	C-16	65.6	C-12, 13, 16
15	74.2	C-13,14,16	74.4	C-14, 16	74.8	C-13, 14, 16
16	169.8		170.1		171.1	
17	108.4	C-7,8,9	109.9	C-7, 9	110.9	C-7, 8
18	28.2	C-3, 4, 5, 19	36.5	C-3, 4, 5, 19	27.8	C-3, 4, 5, 18
19	15.3	C-3, 4, 5, 18	22.3	C-3, 4, 5, 18	15.1	C-3, 4, 5, 19
20	14.3	C-1, 5, 9, 10	15.9	C-1, 5, 9, 10	13.2	C-1, 5, 9, 10

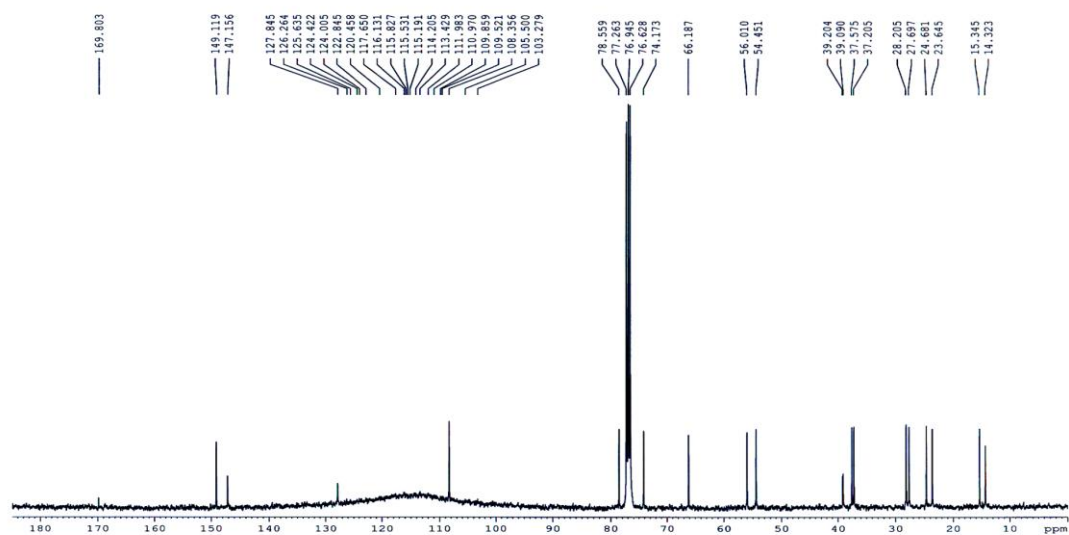
^{a,b} Assignments were based on DEPT, HMQC, and HMBC experiments; chemical shifts are given in ppm.



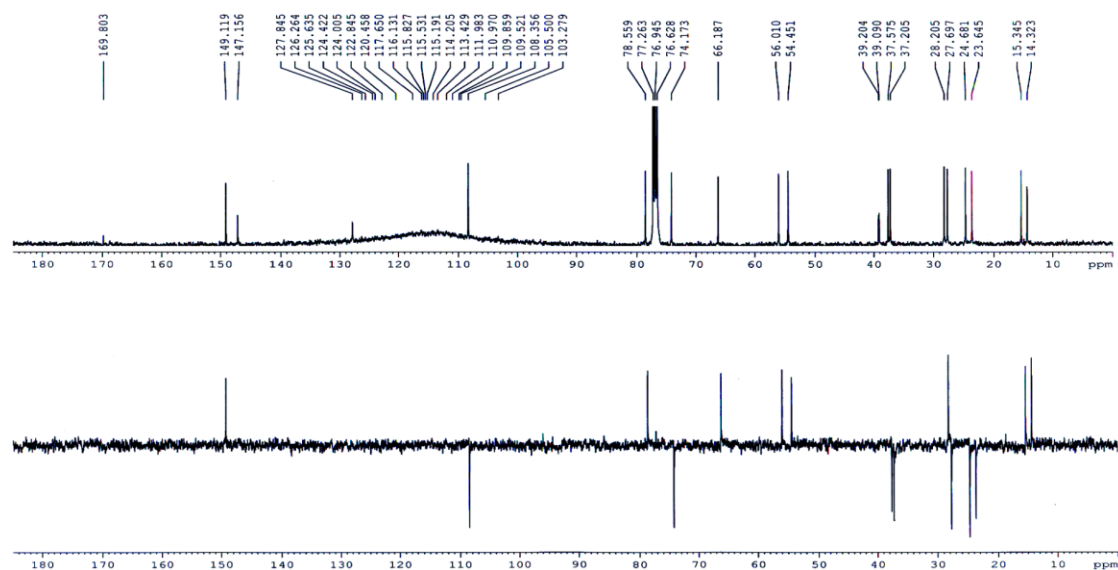
S1. Selected NOE correlations for 3 β -Hydroxyisocoronarin D (**2**)



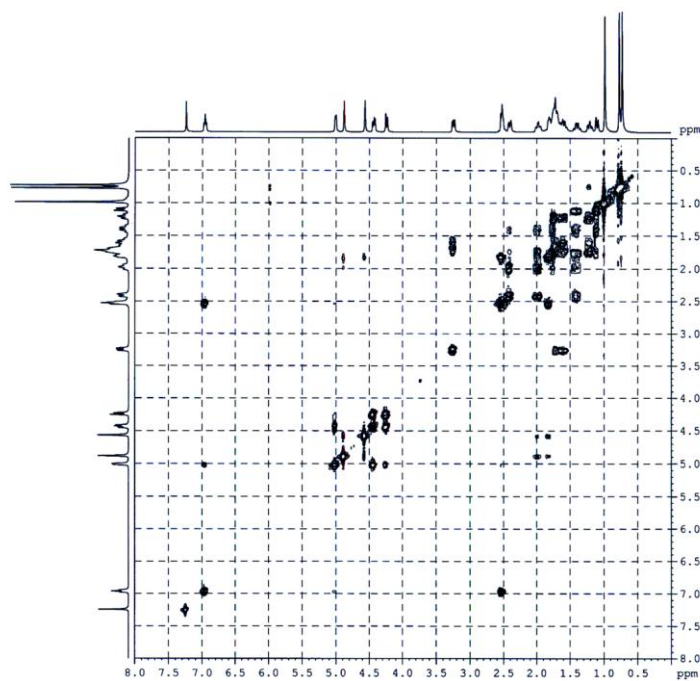
S2. ^1H NMR (CDCl_3 , 400 MHz) spectrum of 3β -Hydroxyisocoronarin D (**2**)



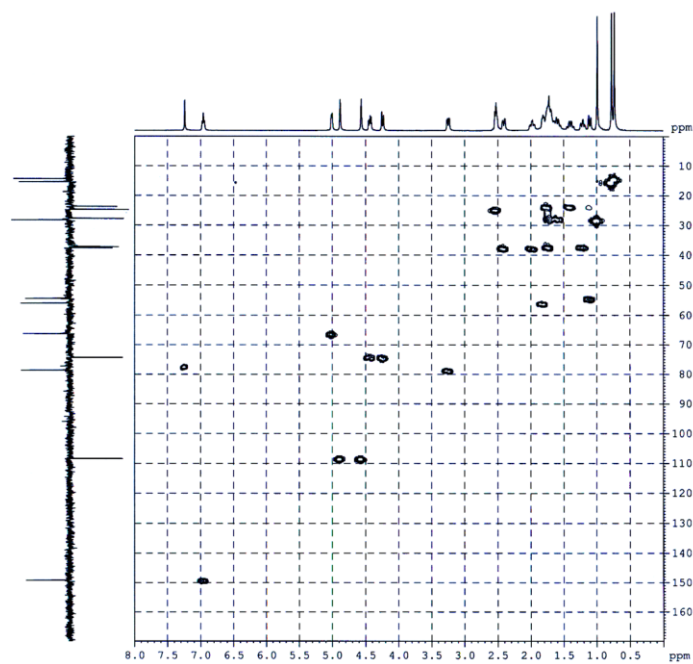
S3. ^{13}C NMR (CDCl_3 , 100 MHz) spectrum of 3β -Hydroxyisocoronarin D (**2**)



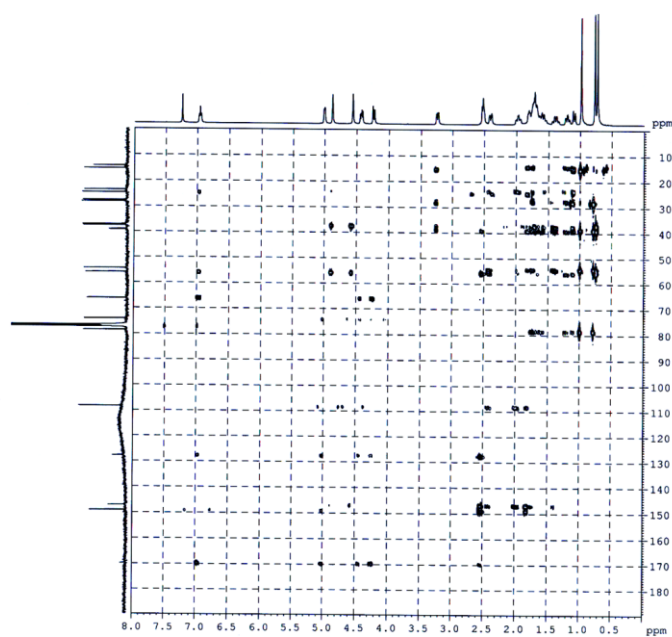
S4. DEPT-135 spectrum of 3 β -Hydroxyisocoronarin D (2)



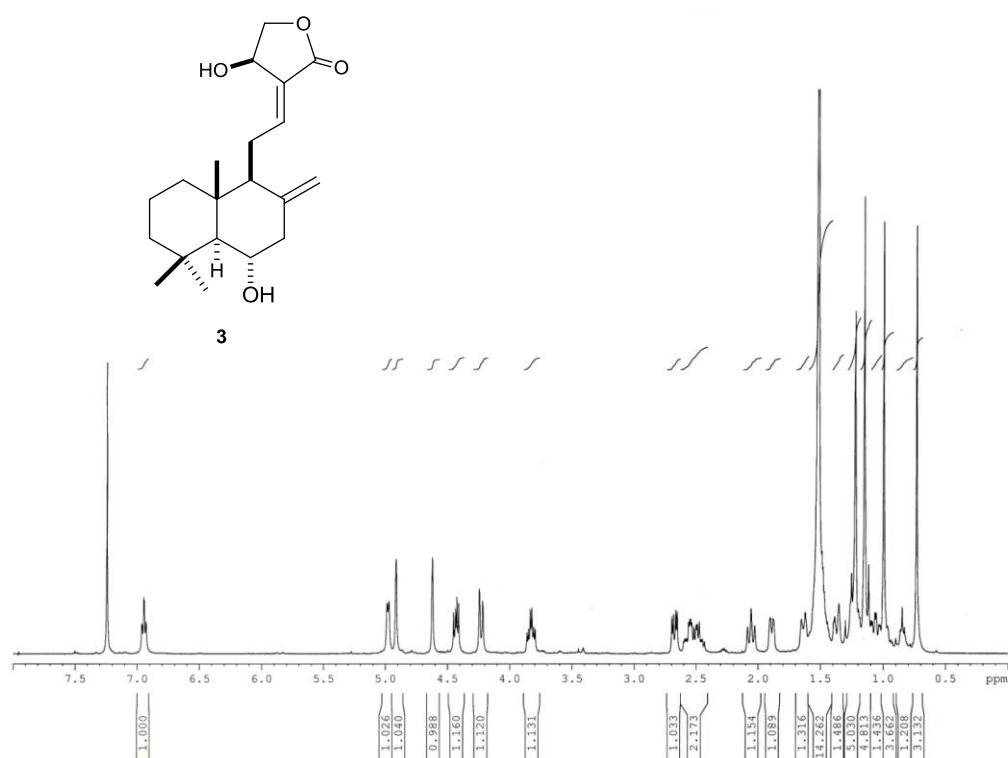
S5. COSY spectrum of 3 β -Hydroxyisocoronarin D (2)



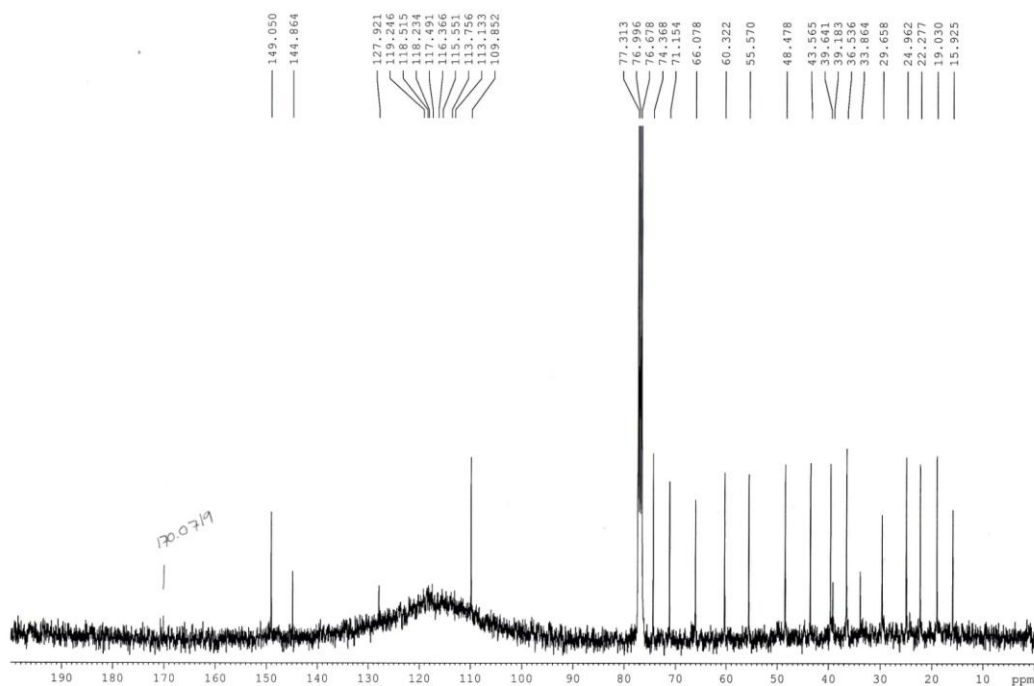
S6. HMQC spectrum of 3 β -Hydroxyisocoronarin D (2)



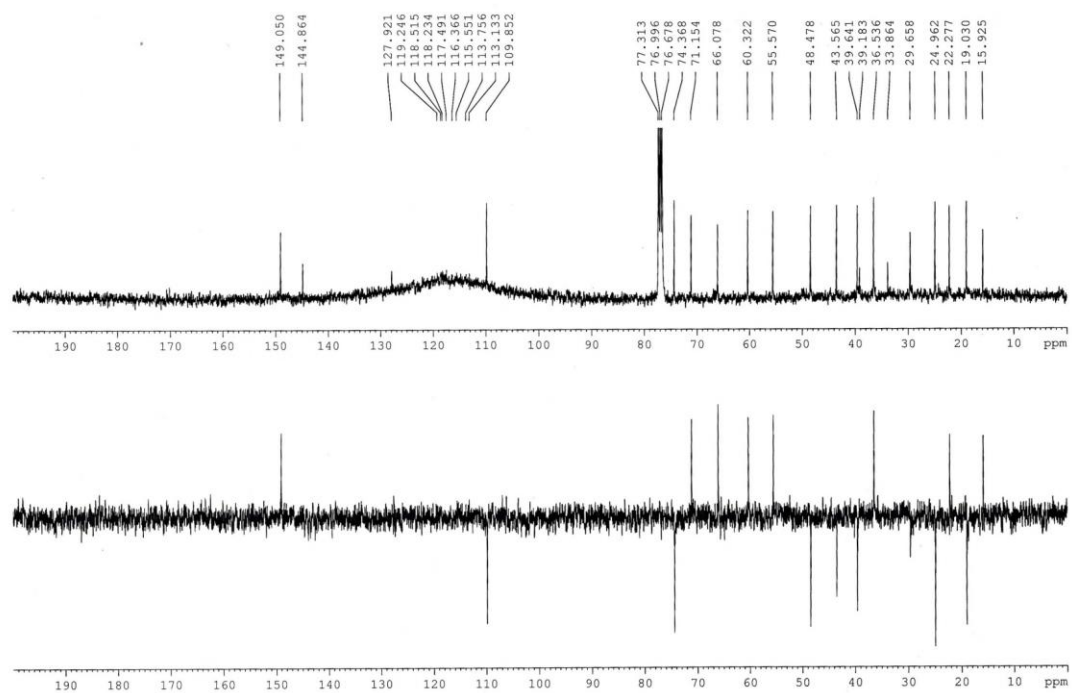
S7. HMBC spectrum of 3 β -Hydroxyisocoronarin D (2)



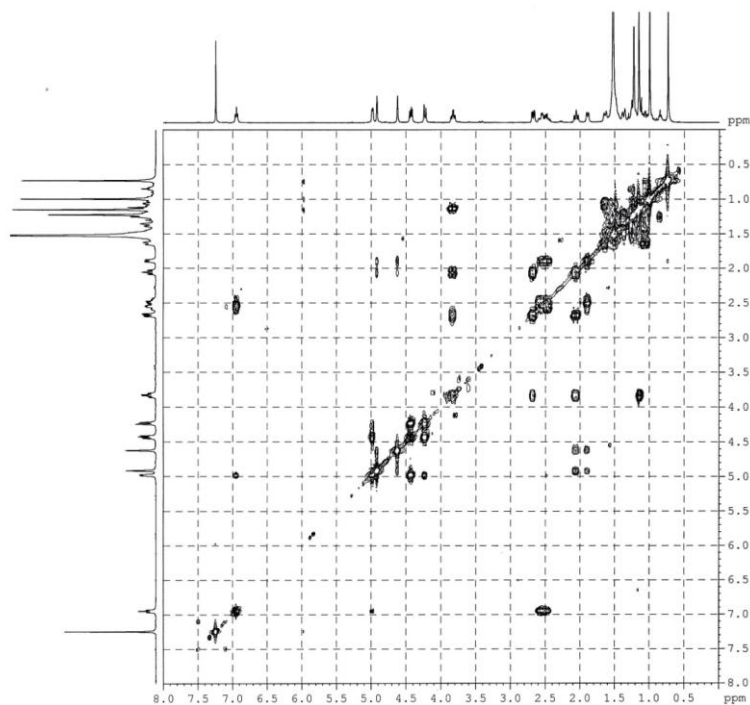
S8. ¹H NMR (CDCl₃, 400 MHz) spectrum of 6 α -Hydroxyisocoronarin D (**3**)



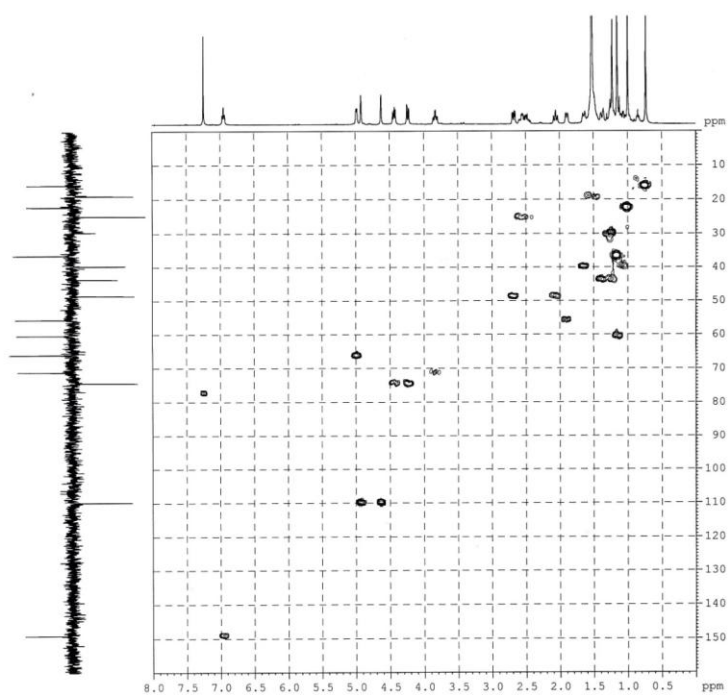
S9. ¹³C NMR (CDCl₃, 100 MHz) spectrum of 6 α -Hydroxyisocoronarin D (**3**)



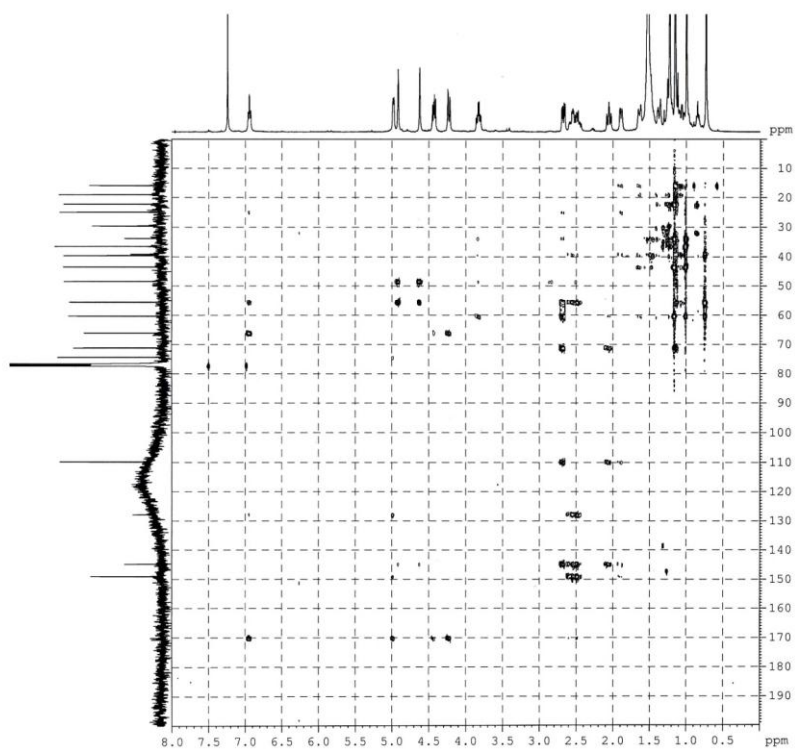
S10. DEPT-135 spectrum of 6 α -Hydroxyisocoronarin D (3)



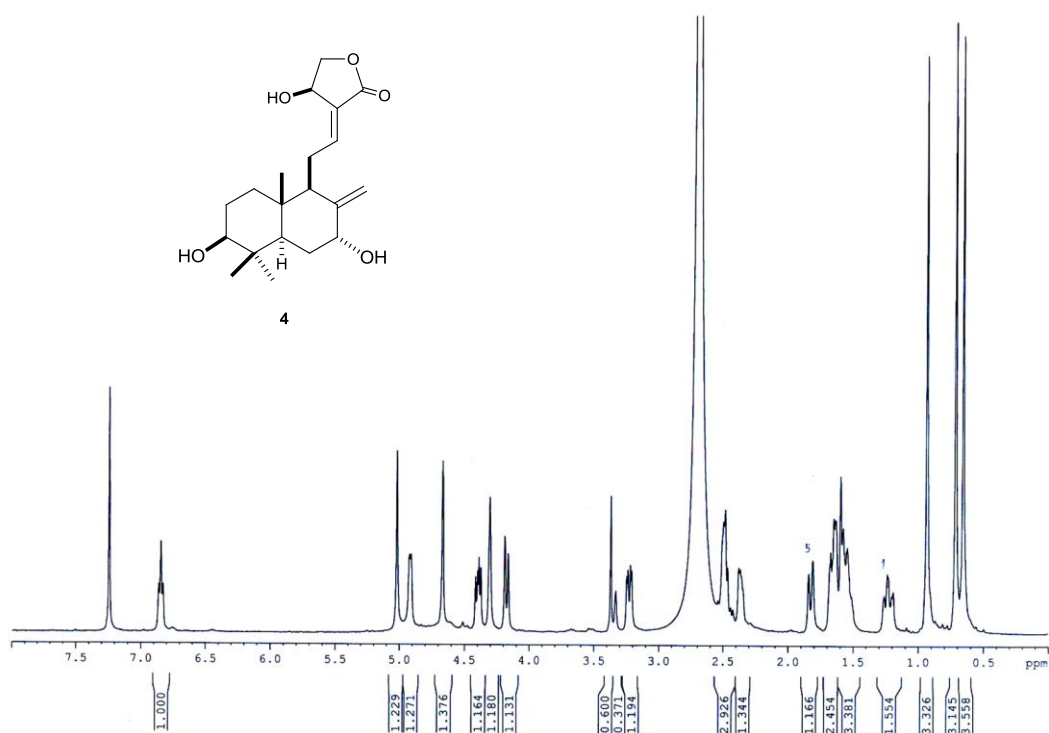
S11. COSY spectrum of 6 α -Hydroxyisocoronarin D (3)



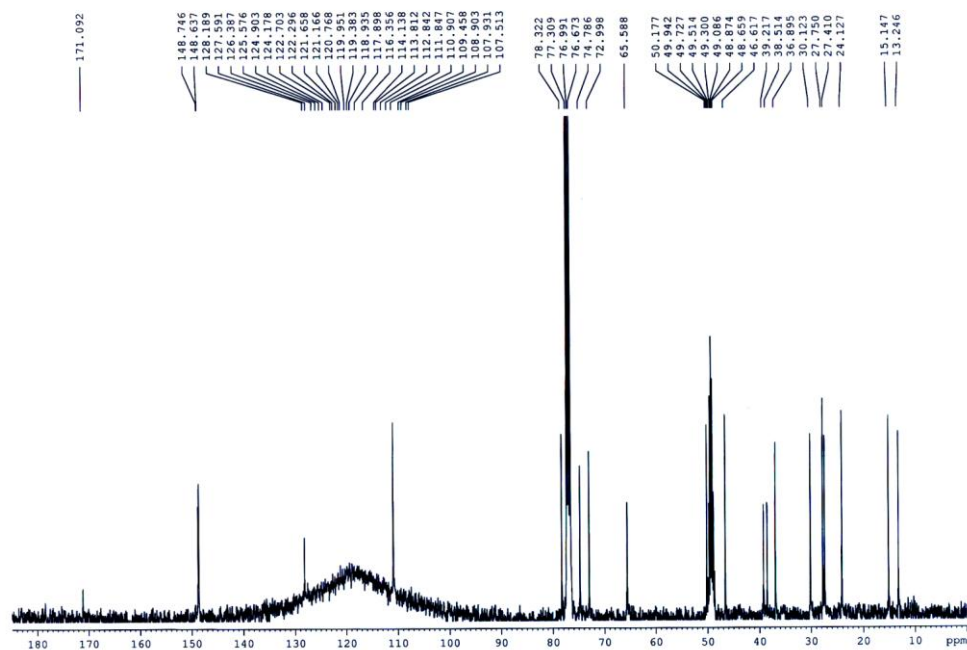
S12. HMQC spectrum of 6 α -Hydroxyisocoronarin D (3)



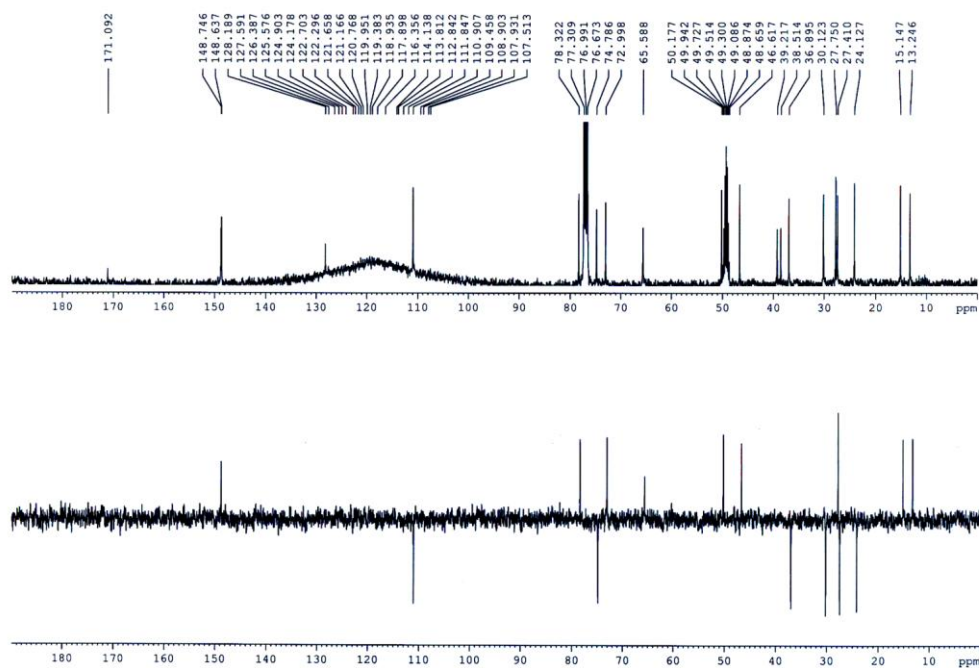
S13. HMBC spectrum of 6 α -Hydroxyisocoronarin D (3)



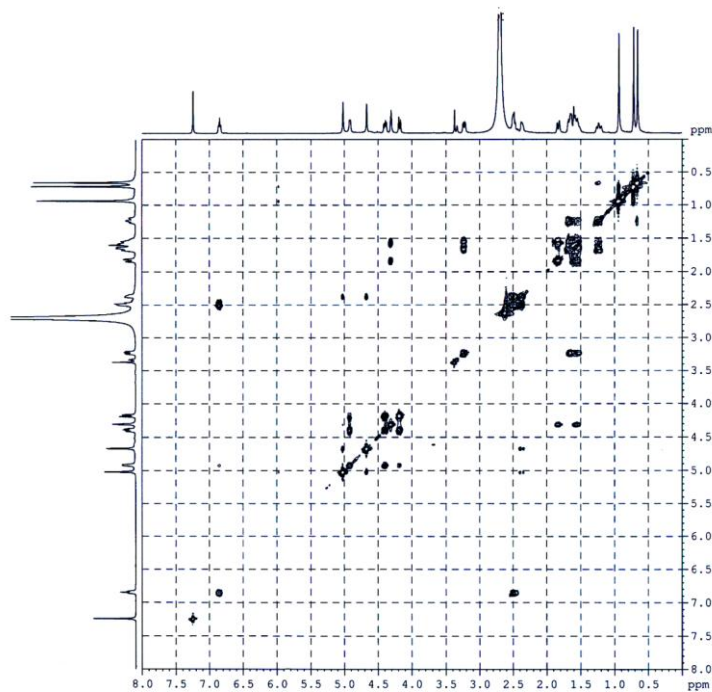
S14. ¹H NMR (CDCl₃, 400 MHz) spectrum of 3β,7α-Dihydroxyisocoronarin D (4)



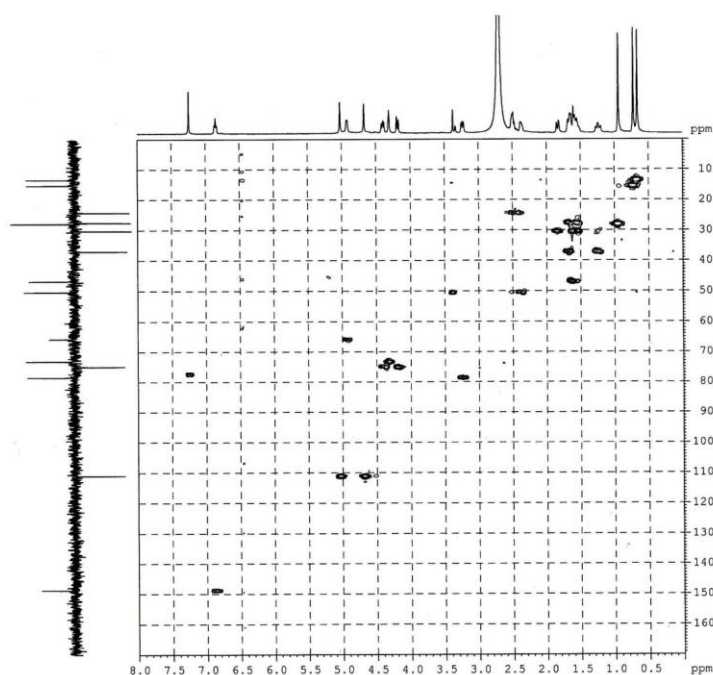
S15. ¹³C NMR (CDCl₃, 100 MHz) spectrum of 3β,7α-Dihydroxyisocoronarin D (4)



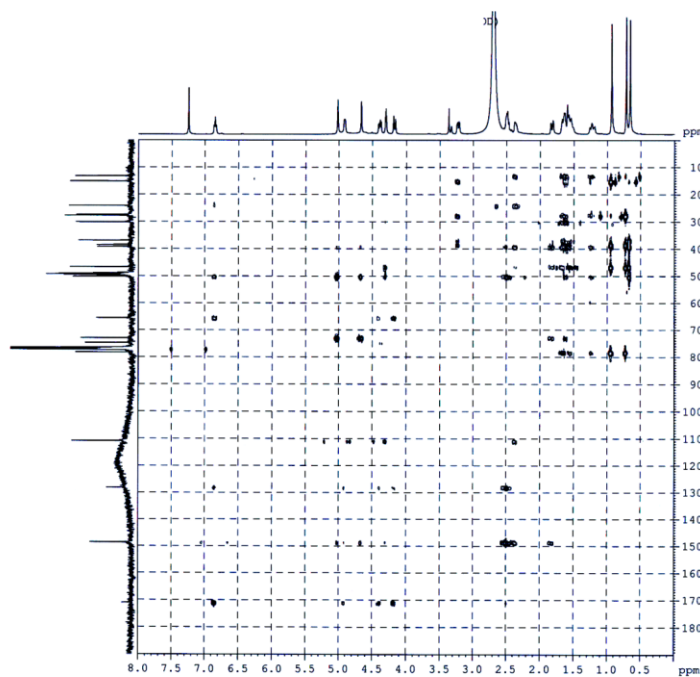
S16. DEPT-135 spectrum of 3β,7α-Dihydroxyisocoronarin D (4)



S17. COSY spectrum of 3β,7α-Dihydroxyisocoronarin D (4)



S18. HMBC spectrum of 3 β ,7 α -Dihydroxyisocoronarin D (**4**)



S19. HMBC spectrum of 3 β ,7 α -Dihydroxyisocoronarin D (**4**)