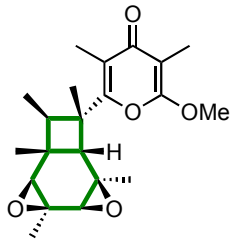
Plakortinic Acids A and B: Cytotoxic Cycloperoxides with a Bicyclo[4.2.0]octene Unit from Sponges of the Genera *Plakortis* and *Xestospongia*

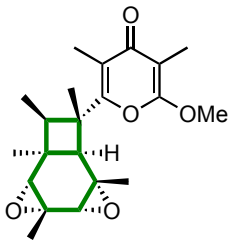
Carlos Jiménez-Romero, Abimael D. Rodríguez, and Sangkil Nam

Supporting Information

Scheme S1. Previously Reported Compounds Possessing a Bicyclo-[4.2.0]octane Core (Green Bold Line).



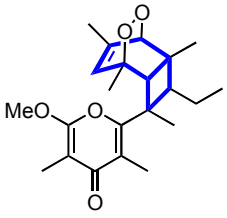
Elysiapyrone A[1]



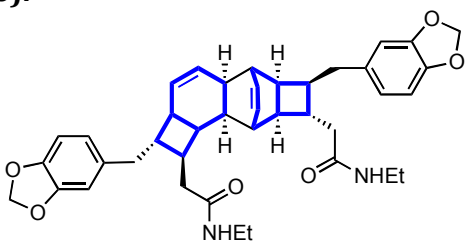
Elysiapyrone B[1]

1. Cueto, M.; D'Croz, L.; Maté, J. L.; San-Martín, A.; Darias, J. *Org. Lett*. **2005**, *7*, 415–418.

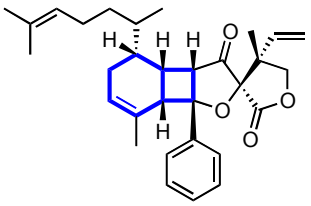
Scheme S2. Previously Reported Compounds Possessing a Bicyclo-[4.2.0]octene Core (Blue Bold Line).



Ocellapyrone B[1]

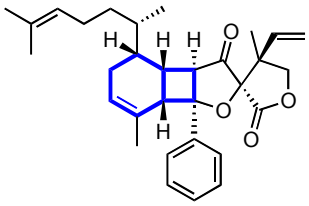


Kingianin A[2]



Biyouyanagin A[3]

anti-HIV activity

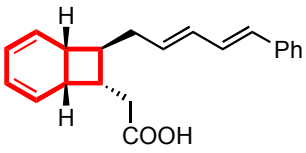


Biyouyanagin B[4]

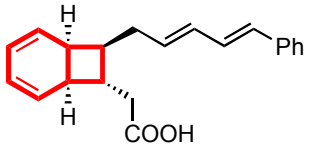
cytotoxic activity

1. Manzo, E.; Ciavatta, M. L.; Gavagnin, M.; Mollo, E.; Wahidulla, S.; Cimino, G. *Tetrahedron Lett*. **2005**, *46*, 465–468.
2. Leverrier, A.; Elise, M.; Dau, T. H.; Retailleau, P.; Awang, K.; Guéritte, F.; Litaudon, M. *Org. Lett*. **2010**, *12*, 3638–3641.
3. Tanaka, N.; Okasaka, M.; Ishimaru, Y.; Takaishi, Y.; Sato, M.; Okamoto, M.; Oshikawa, T.; Ahmed, S. U.; Consentino, L. M.; Lee, K-H. *Org. Lett*. **2005**, *7*, 2997–2999.
4. Tanaka, N.; Kashiwada, Y.; Kim, S. Y.; Hashida, W.; Sekiya, M.; Ikeshiro, Y.; Takaishi, Y. *J. Nat. Prod*. **2009**, *72*, 1447–1452.

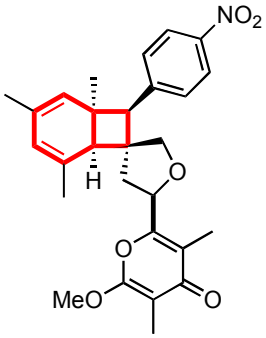
Scheme S3. Previously Reported Compounds Possessing a Bicyclo-[4.2.0]octadiene core (Red Bold Line).



Endiandric acid D[1]

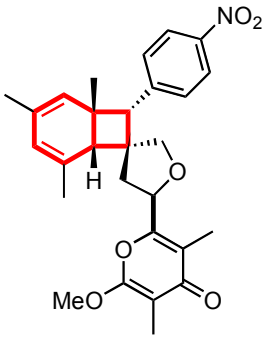


Endiandric acid E[1]



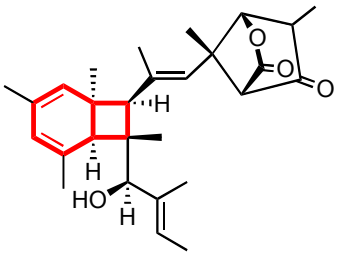
SNF4435C[2]

immunosuppressants



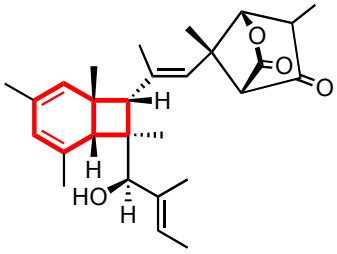
SNF4435D[2]

immunosuppressants



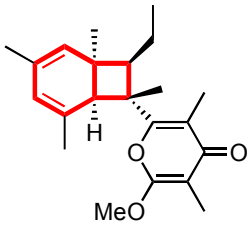
Shimalactone A[3]

cytotoxic activity

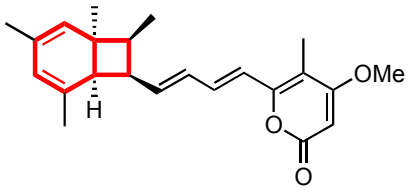


Shimalactone B[4]

cytotoxic activity



Ocellapyrone A[5]



Citreoviripyrone A[6]

inhibitory activity on cell growth

1. Banfield, J. E.; Black, D. St. C.; Johns, S. R.; Willing, R. I. *Aust. J. Chem*. **1982**, *35*, 2247–2256.
2. (a) Kurosawa, K.; Takahashi, K.; Tsuda, E. *Antibiot*. **2001**, *54*, 541–547. (b) Takahashi, K.; Tsuda, E.; Kurosawa, K. *Antibiot*. **2001**, *54*, 548–553.
3. Wei, H.; Itoh, T.; Kinoshita, M.; Kotoku, N.; Aoki, S.; Kobayashi, M. *Tetrahedron* **2005**, *61*, 8054–8058.
4. Wei, H.; Itoh, T.; Kotoku, N.; Kobayashi, M. *Heterocycles* **2006**, *68*, 111–123.
5. (a) Manzo, E.; Ciavatta, M. L.; Gavagnin, M.; Mollo, E.; Wahidulla, S.; Cimino, G. *Tetrahedron Lett*. **2005**, *46*, 465–468. (b) Miller, A. K.; Trauner. D. *Angew. Chem., Int. Ed*. **2005**, *44*, 4602-4606.
6. Asai, T.; Luo, D.; Yamashita, K.; Oshima, Y. *Org. Lett*. **2013**, *15*, 1020–1023.

Table S1. ^{1}H–^{1}H COSY, HMBC (H → C#) and NOESY NMR Data of Plakortinic Acids (2 and 3) in $CDCl_3$

Position	^{1}H–^{1}H COSY	HMBC (H → C#)	NOESY
1			
2α	H-2β	1, 3, 4, 22	H-4*β*, H_3-22
2*β*	H-2α	1, 3, 4, 22	H-4*β*, H_3-23
3			
4α	H-4β	2, 3, 5, 6, 7, 22, 23	H-4β, H-6α*β*, H_3-22
4*β*	H-4α	2, 3, 5, 6, 22, 23	H-2α*β*, H-4α, H_3-23
5			
6α		7	H-4α
6*β*		7	H-4α
7α			
7*β*			
8			
9			
10α			
10β			
11α	H-12	9, 12	H-14
11*β*	H-12	9, 12	H-14
12	H-11, H-13, H-19	20	H-13, H_3-21
13	H-12, H-14, H-18	12, 14, 18, 19	H-12
14	H-13, H-15	12, 13	H-11α*β*, H-15, H-19
15	H-14, H-16	13, 14, 16, 17	H-14, H-16, H-19
16	H-15, H-24	14, 15, 18, 24	H-15, H_3-24
17			
18	H-13, H-19	13, 14, 16, 17, 19, 20, 24	H_3-21
19	H-12, H-18, H-20	12, 18, 21	H-14, H_3-21
20α	H-19, H-21	12, 18, 19, 21	
20β	H-21	12, 18, 19, 21	
21	H-20	19, 20	H-12, H-18, H-19, H_3-24
22		2, 3, 4	H-2α, H-4α
23		4, 5, 6, 7	H-2β, H-4*β*
24	H-16	16, 17, 18	H-16, H_3-21

Figure S1. a) Key $^{1}H–^{1}H$ COSY and HMBC correlations. **b**) NOESY correlations for plakortinic acids A and B (**2** is shown).

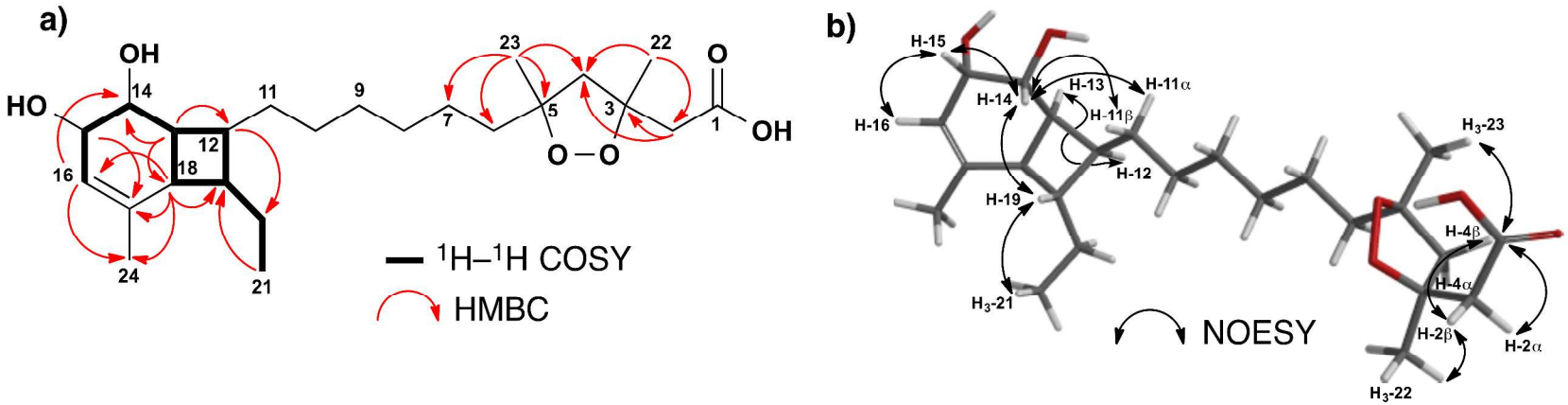
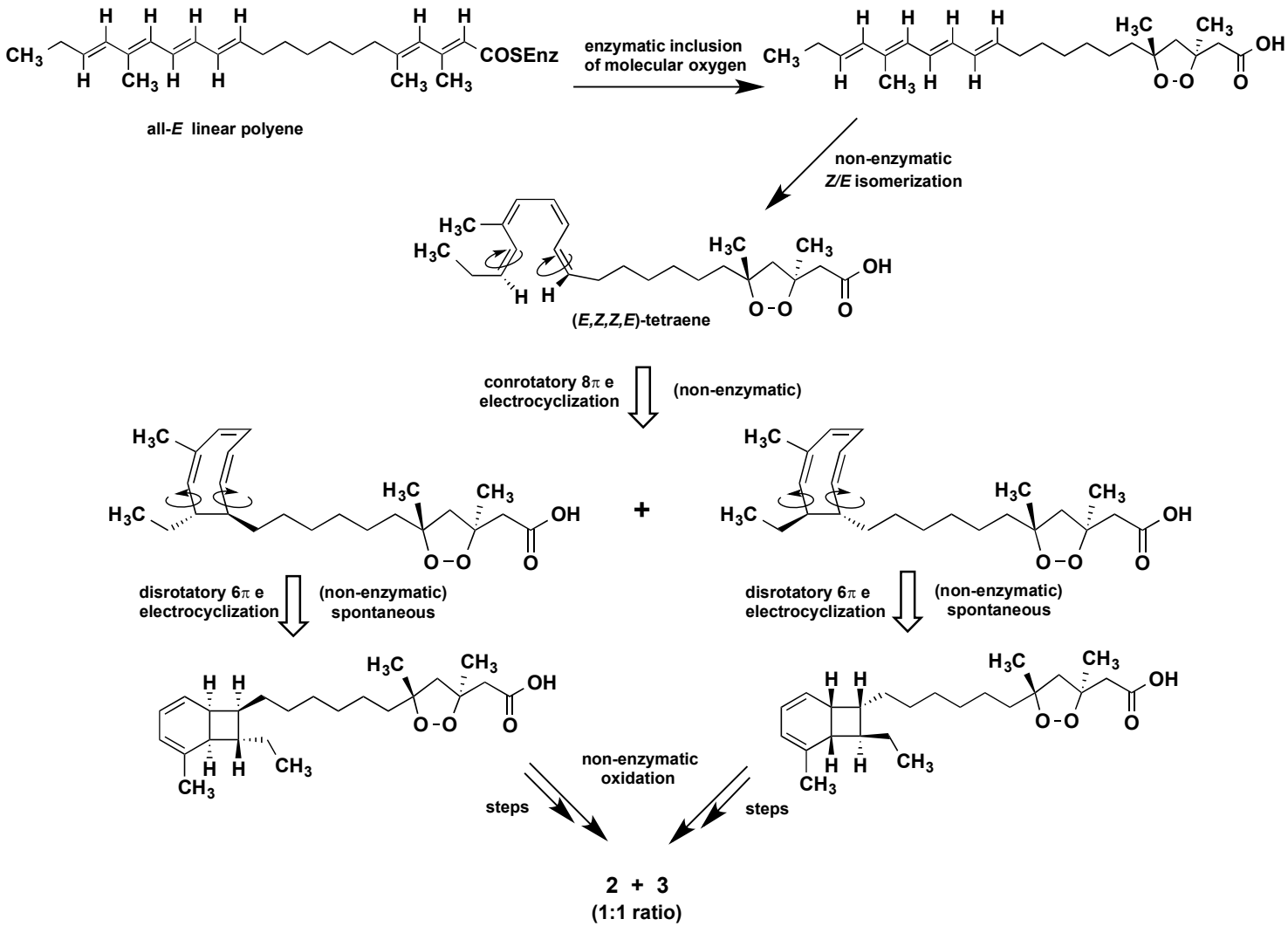


Figure S2. Plausible biogenetic pathway for plakortinic acid A (**2**) and plakortinic acid B (**3**).



Experimental Section

General Experimental Procedures. Optical rotations were obtained with an Autopol IV automatic polarimeter. Infrared spectra were obtained with a Nicolet Magna FT-IR 750 spectrometer. 1D- and 2D-NMR spectra were recorded with a Bruker DRX-500 FTNMR spectrometer. Mass spectrometric data were generated at the Mass Spectrometry Laboratory of the University of Illinois at Urbana-Champaign. Column chromatography (CC) was performed using silica gel (35–75 mesh). TLC analysis was carried out using glass pre-coated silica gel plates, and the spots were visualized by exposure to I_2 vapor. All solvents used were either spectral grade or distilled from glass prior to use. Commercially available Diazald® and dimolybdenum tetraacetate were purchased from Sigma Aldrich Co.

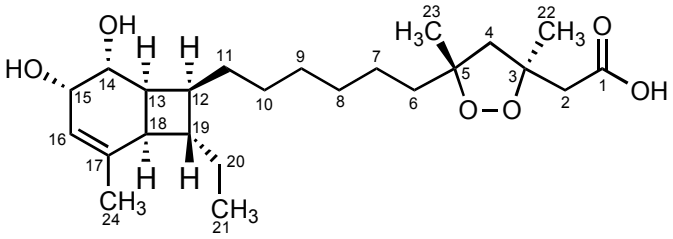
Animal Material. All individuals, found along the ceiling of cave overhangs with a drop shape, were massively encrusting with irregular conulose surface. Most specimens collected measured up to 20 cm long and 5 cm thick. All *Plakortis halichondrioides* colonies were overgrown with the thinly-encrusting sponge *Xestospongia deweerdtae*, which provides a lavender pink crust over the olive green color of *P. halichondrioides*. However, specimens turned to a brownish color, producing a dark exudate when brought to the surface. Individuals were easily broken, with firm consistency. Noticeable oscules along the surface were circular and measured 2.0–10.0 mm in diameter. The choanosome was compact with many cavities. Both choanosome and ectosome formed by high abundance of diods that were arranged homogenously and densely over the sponge body. Diods were curved with sharp edges. Straight triods with sharp edges were highly abundant. Nonetheless, many triods had rounded edges with a thick center. Triods and diods were variable in size. Minimum diod length varied from 110 to 160 μm, and the maximum actine length of triod length varied between 20 and 60 μm. The ectosome measured between 450 and 650 μm thick. A high density of unusual cavities formed a mesh that ran perpendicular to the surface of the ectosome. An underwater photograph of one of the sponge specimens is included as part of Supporting Information.

Collection, Extraction, and Isolation. Fresh specimens of the sponge *Plakortis halichondrioides* (Wilson, 1902) (phylum Porifera; class Demospongiae; subclass Homoscleromorpha; order Homosclerophorida; family Plakinidae) were collected by hand

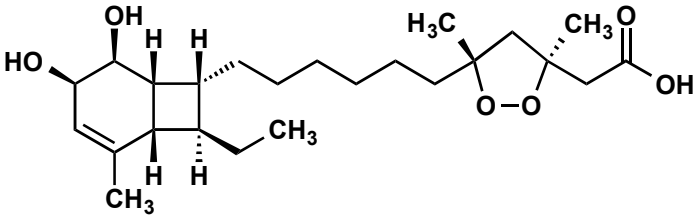
using scuba at depths of 90–100 ft off Mona Island, Puerto Rico, in July 2006. A voucher specimen (No. IM06-09) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The organism was frozen and lyophilized prior to extraction. The dry specimens (395 g) were cut into small pieces and blended in a mixture of $CHCl_3$–MeOH (1:1) (11 X 1 L). After filtration, the crude extract was concentrated and stored under vacuum to yield a dark gum (100 g), which was suspended in H_2O (2 L) and extracted with *n*-hexane (3 X 2 L), $CHCl_3$ (3 X 2 L), and EtOAc (3 X 2 L). Concentration under reduced pressure yielded 16.4 g of the *n*-hexane extract as a dark brown oil, a portion of which (3.7 g) was chromatographed over silica gel (130 g) using mixtures of *n*-hexane–acetone of increasing polarity (0–100%). A total of 11 fractions (I–XI) were generated on the basis of TLC and ^1H NMR analysis. Further purification of fraction II (1.3 g) by silica gel (20.0 g) column chromatography in 2% acetone–*n*-hexane afforded eight subfractions, denoted as A–H. The most polar fraction H (215 mg) was subjected to CC using reverse-phased silica gel (5 g) and eluted with a MeOH–H_2O gradient (6:4; 7:3; 8:2; 9:1; 1:0) to yield 7 sub-fractions denoted as H1–H7. Subfraction H4 (30 mg) was purified through a short plug of silica gel (0.8 g) with a $CHCl_3$–MeOH gradient (10:0; 9.5:0.5; 9:1) to afford an inseparable mixture in 1:1 ratio of plakortinic acid A (**2**) and plakortinic acid B (**3**) (8.0 mg, 0.01% yield). Purification of subfraction II(H) (659.1 mg) by silica gel (13.0 g) CC using $CHCl_3$ as eluent afforded known epiplakinic acid F (**1**) (170 mg, 0.21% yield).

Cytotoxicity Assays. DU-145 human prostate cancer and A2058 melanoma cell lines were obtained from ATCC. These cells were cultured in RPMI-1640 or DMEM medium containing 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. All cells were maintained in a 5% CO_2 atmosphere at 37 °C. To determine the viability of the cells, Promega CellTiter 96 aqueous nonradioactive cell proliferation assays (MTS) were performed as described by the supplier (Promega; Madison, WI). Briefly, cells (5000/well) were seeded in 96-well plates and incubated overnight at 37 °C in 5% CO_2. Cells were treated for 48 h with each compound. The concentration used was 10 μM. Dimethyl sulfoxide (DMSO) was used as the vehicle control. IC_{50} values of compounds were determined in a dose-dependent manner (0.1, 0.5, 1, 5, 10, 20, and 50 μM). Cell viability was determined by tetrazolium conversion to its formazan dye, and absorbance of formazan was measured at 490 nm using an automated

ELISA plate reader. The production of formazan dye was directly proportional to the number of living cells. Each experiment was done in quadruplicate in the absence of a positive control.

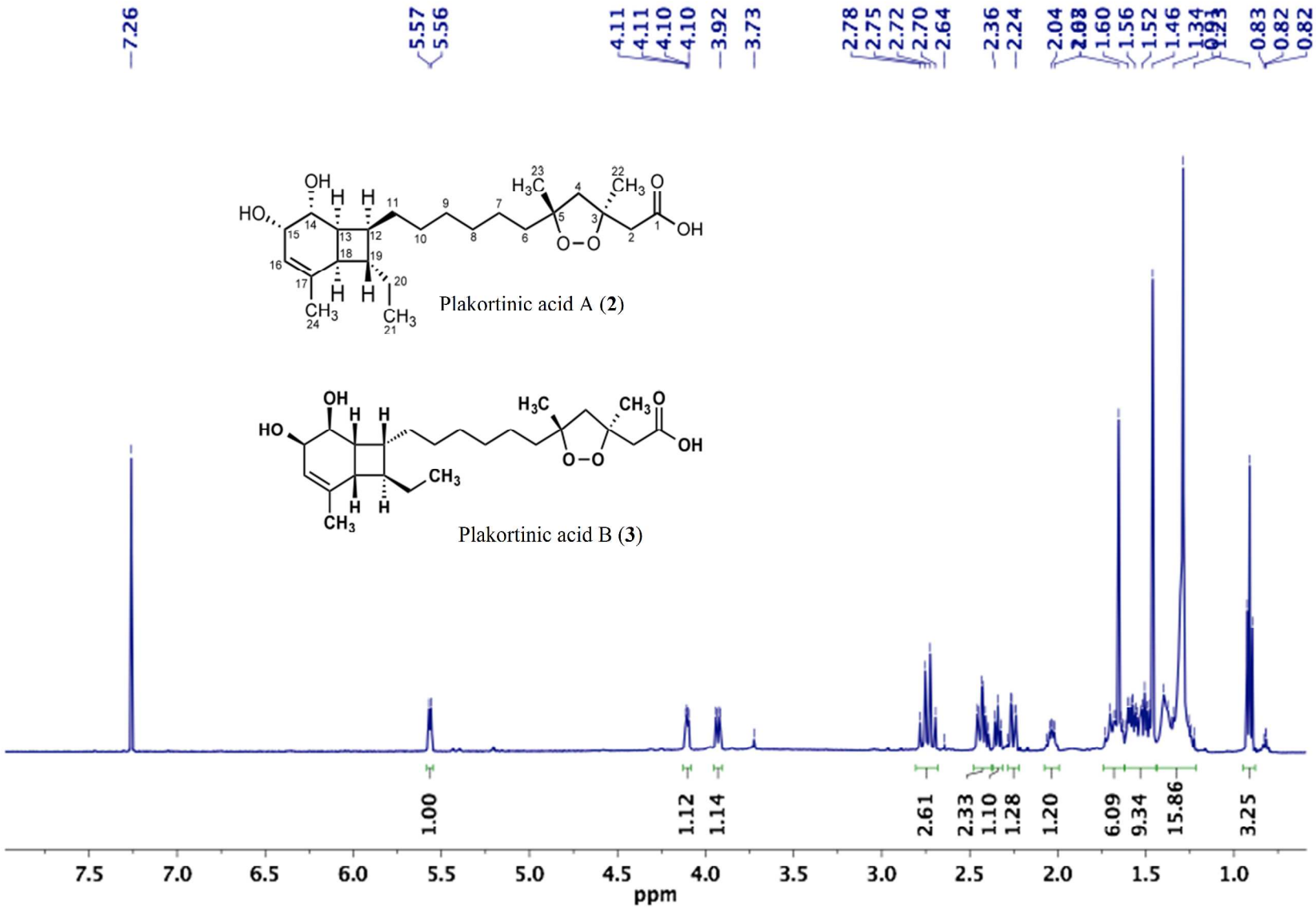


Plakortinic acid A (**2**)

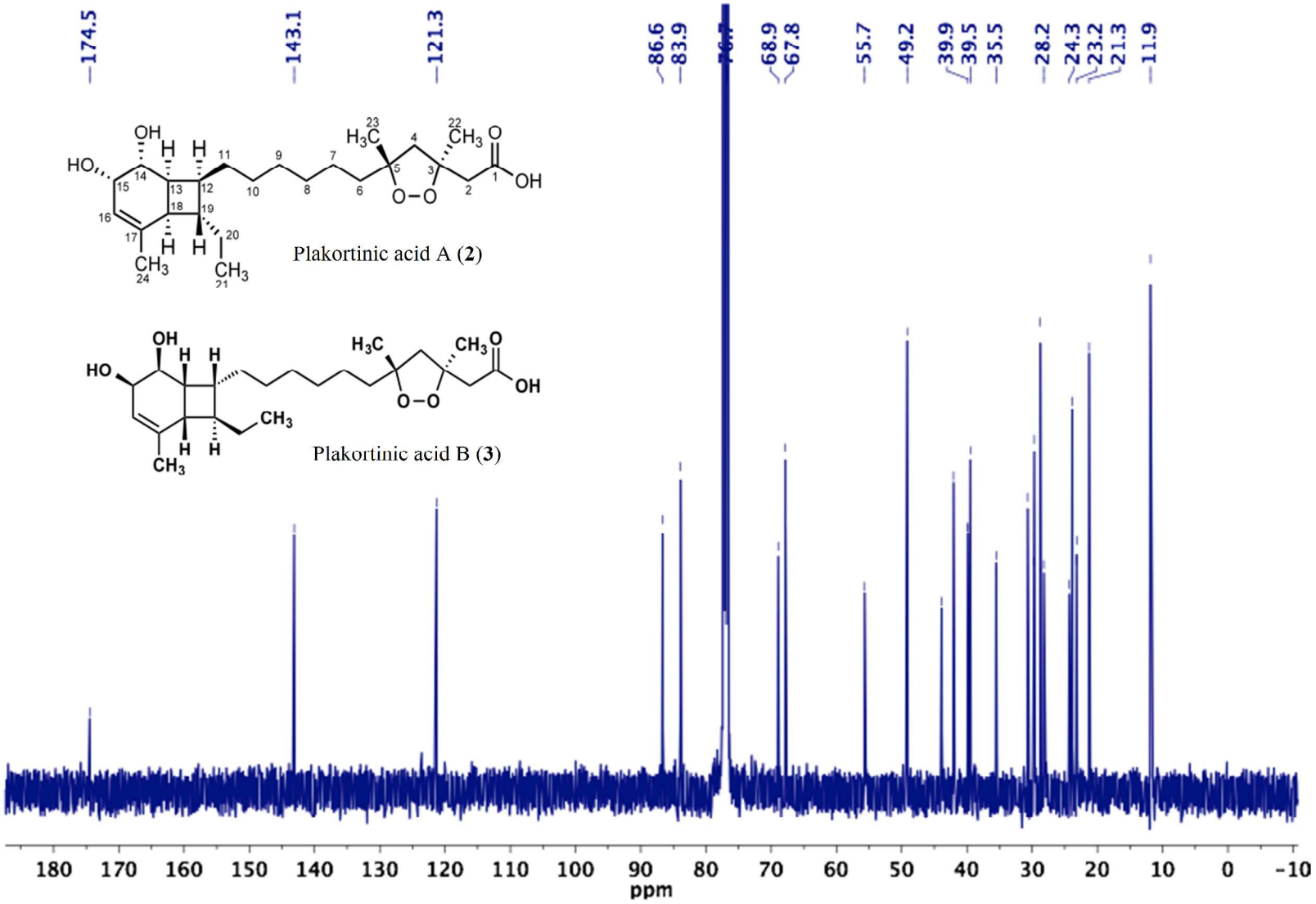


Plakortinic acid B (**3**)

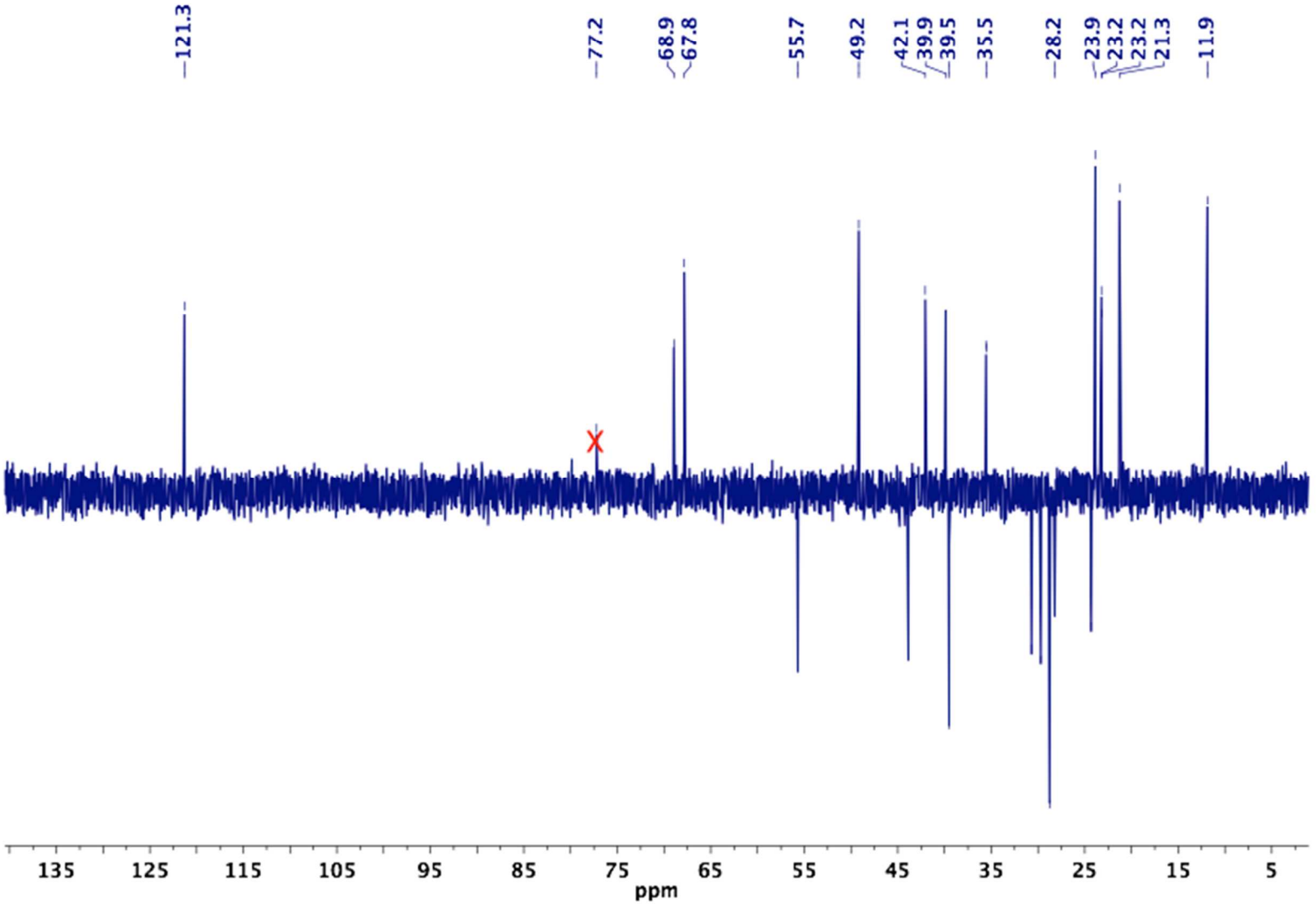
^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of the mixture of plakortinic acids A (**2**) and B (**3**).



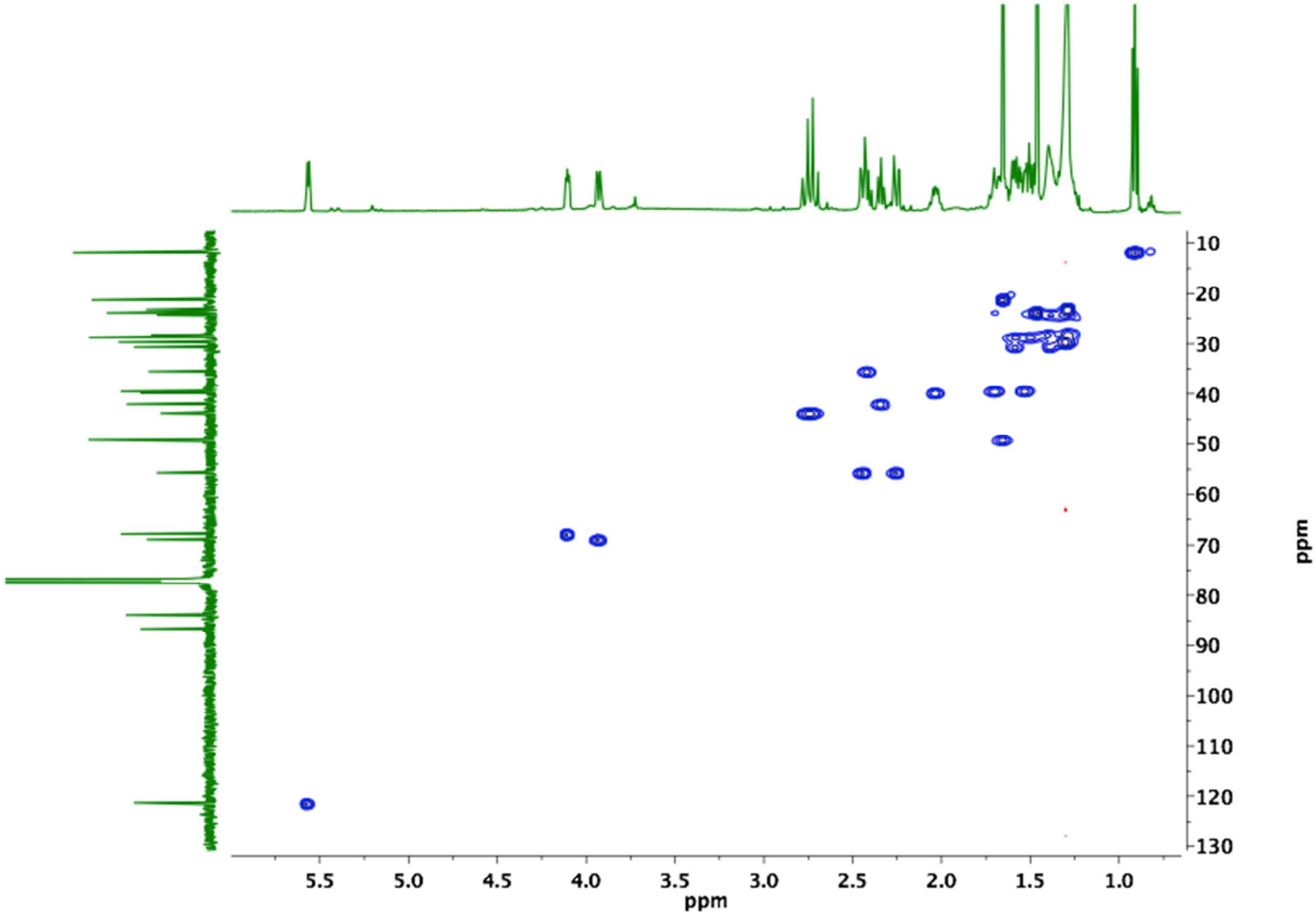
^{13}C-NMR spectrum ($CDCl_3$, 125 MHz) of the mixture of plakortinic acids A (**2**) and B (**3**).



DEPT-135 spectrum ($CDCl_3$, 125 MHz) of the mixture of plakortinic acids A (**2**) and B (**3**).

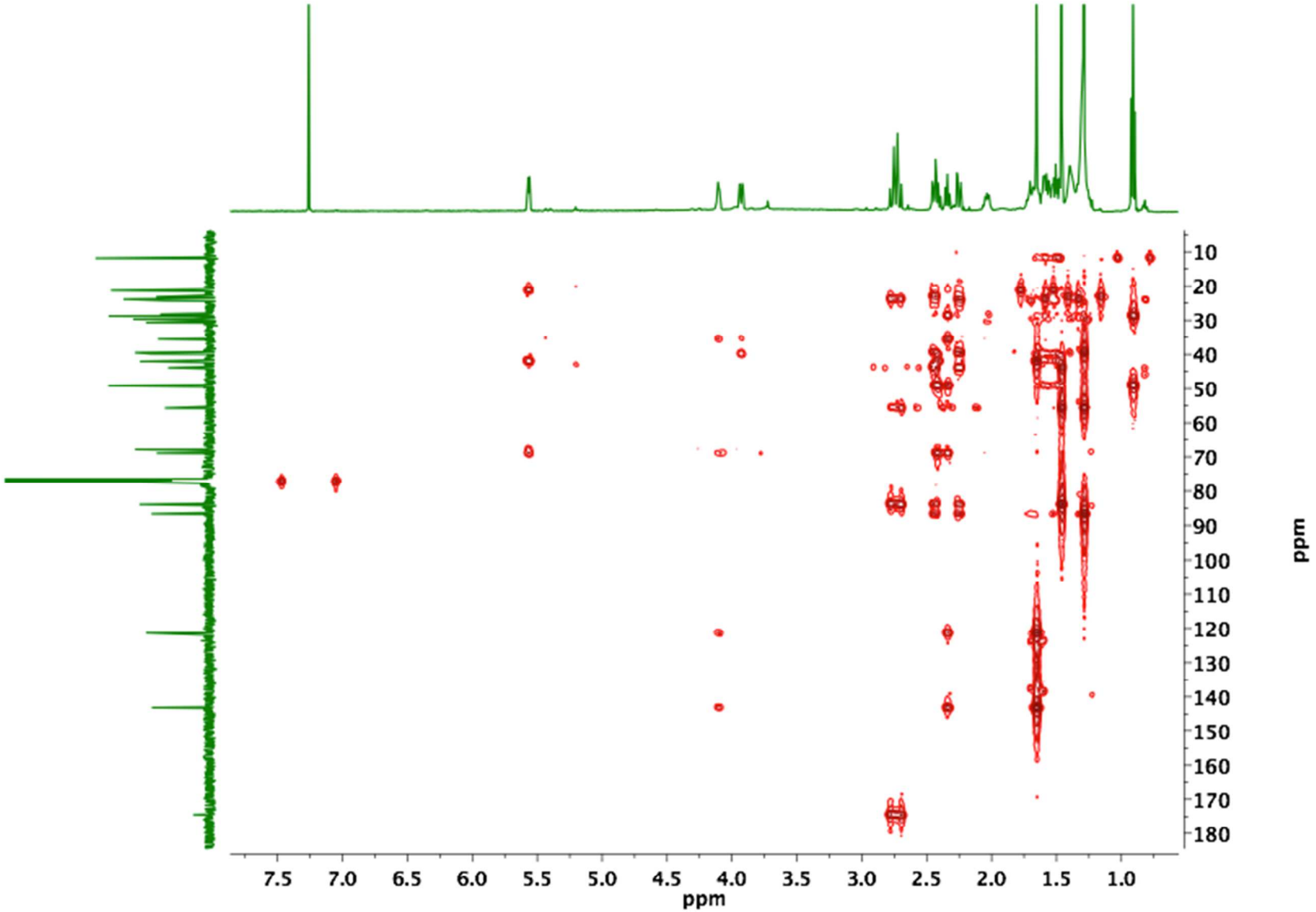


HSQC spectrum (in $CDCl_3$) of the mixture of plakortinic acids A (**2**) and B (**3**).

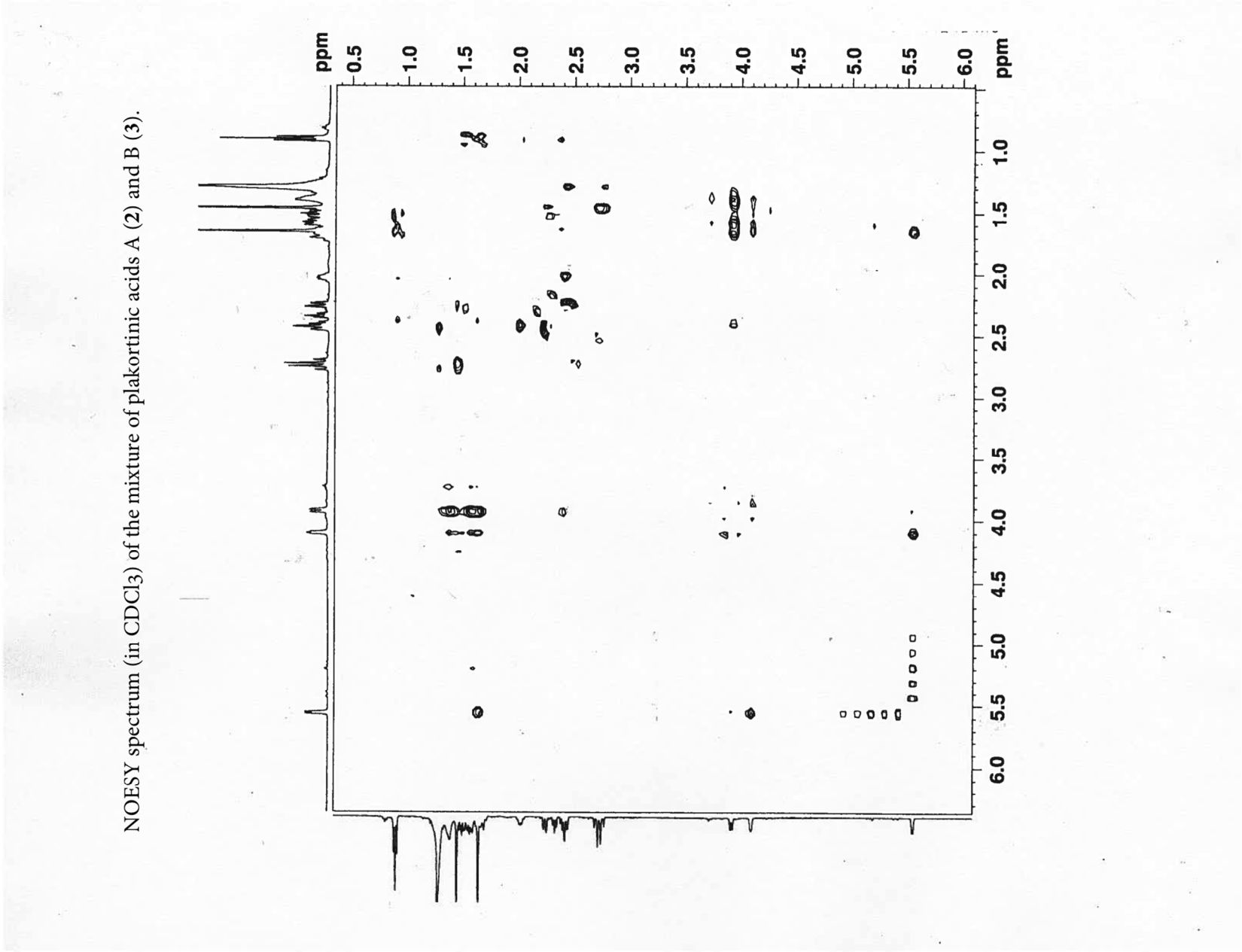


H

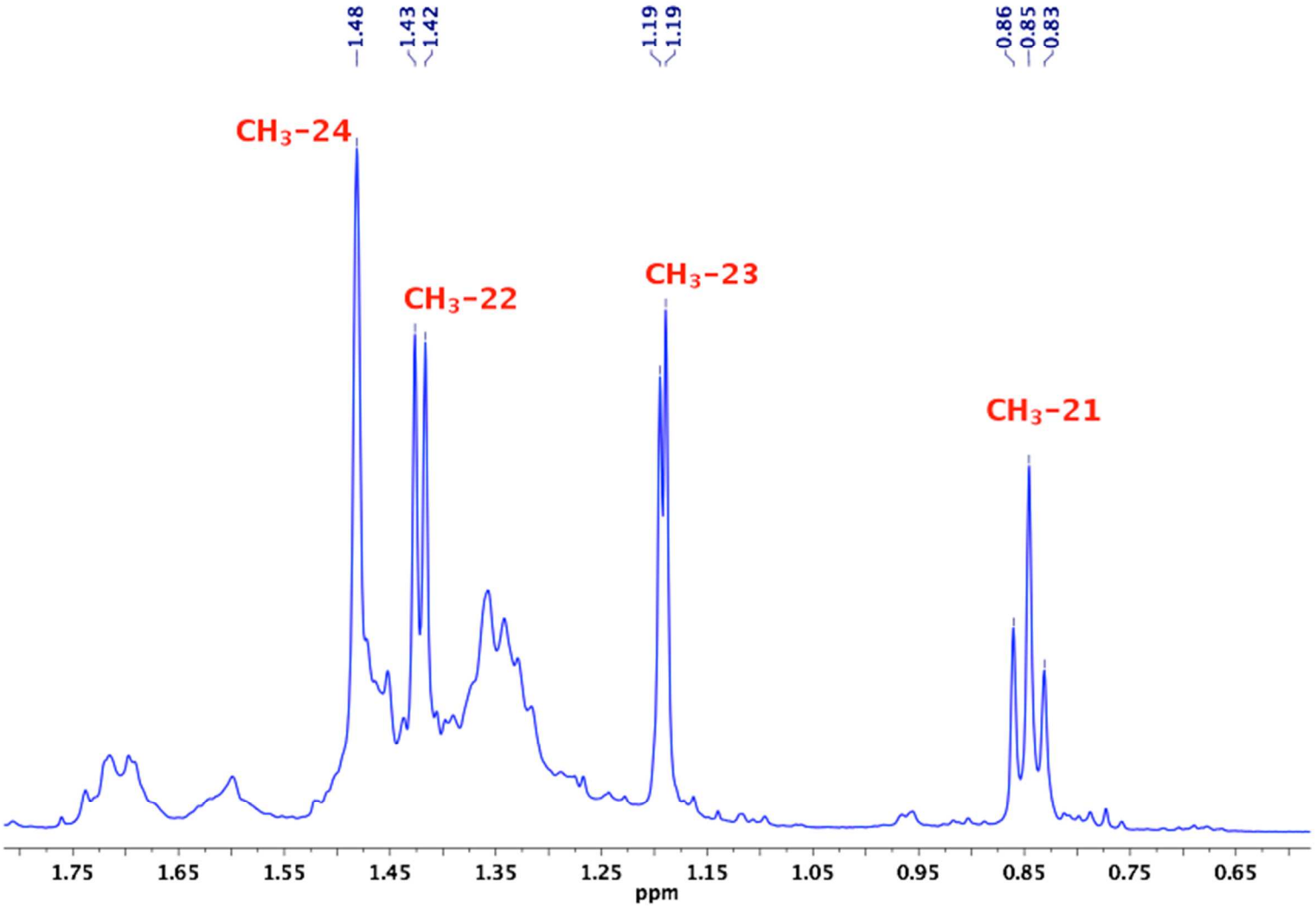
HMBC spectrum (in $CDCl_3$) of the mixture of plakortinic acids A (**2**) and B (**3**).



NOESY spectrum (in $CDCl_3$) of the mixture of plakortinic acids A (**2**) and B (**3**).

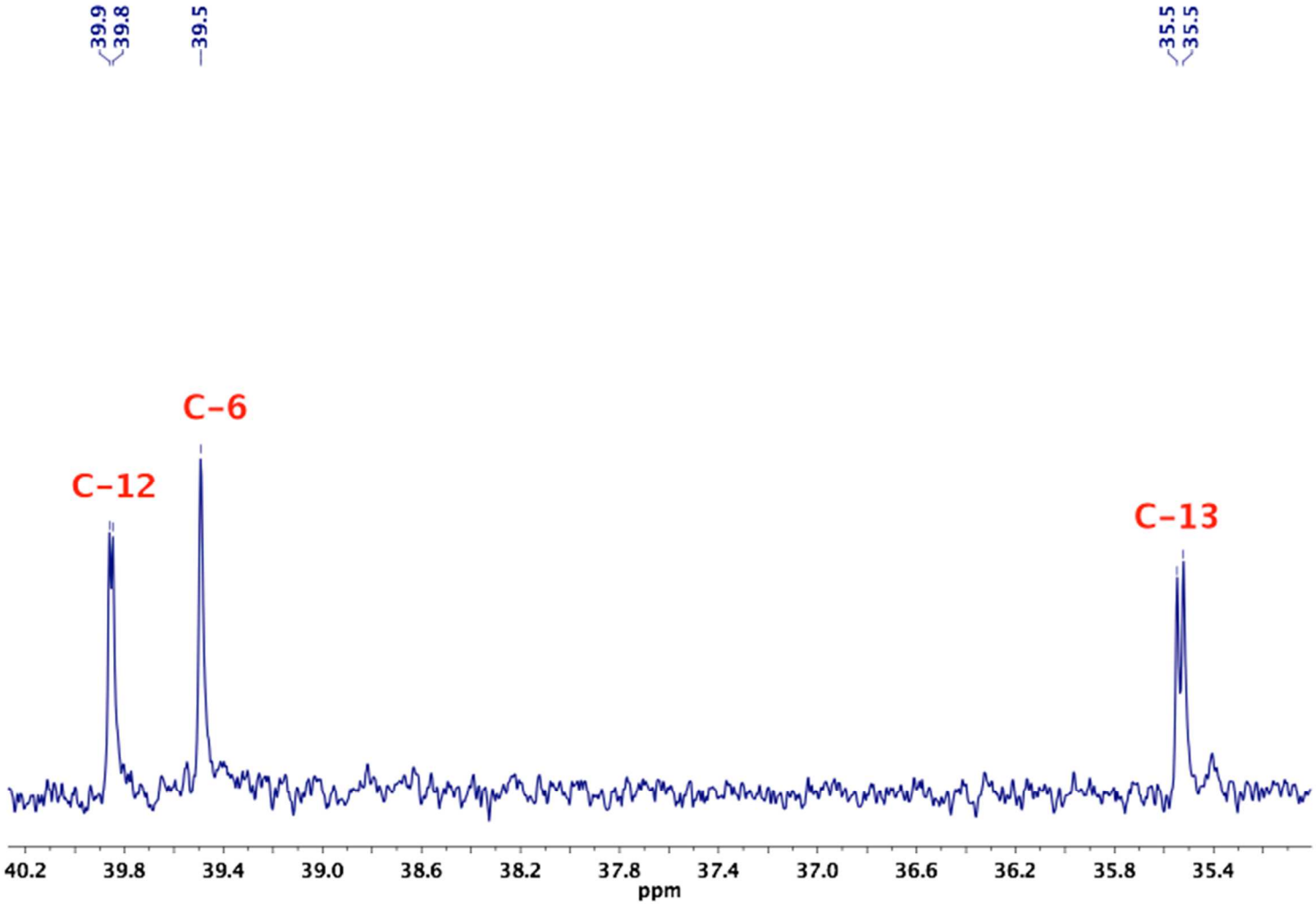


Expansion of the ^{1}H-NMR spectrum (500 MHz) of the mixture of plakortinic acids A (**2**) and B (**3**) in benzene-d_6 (high-field region).



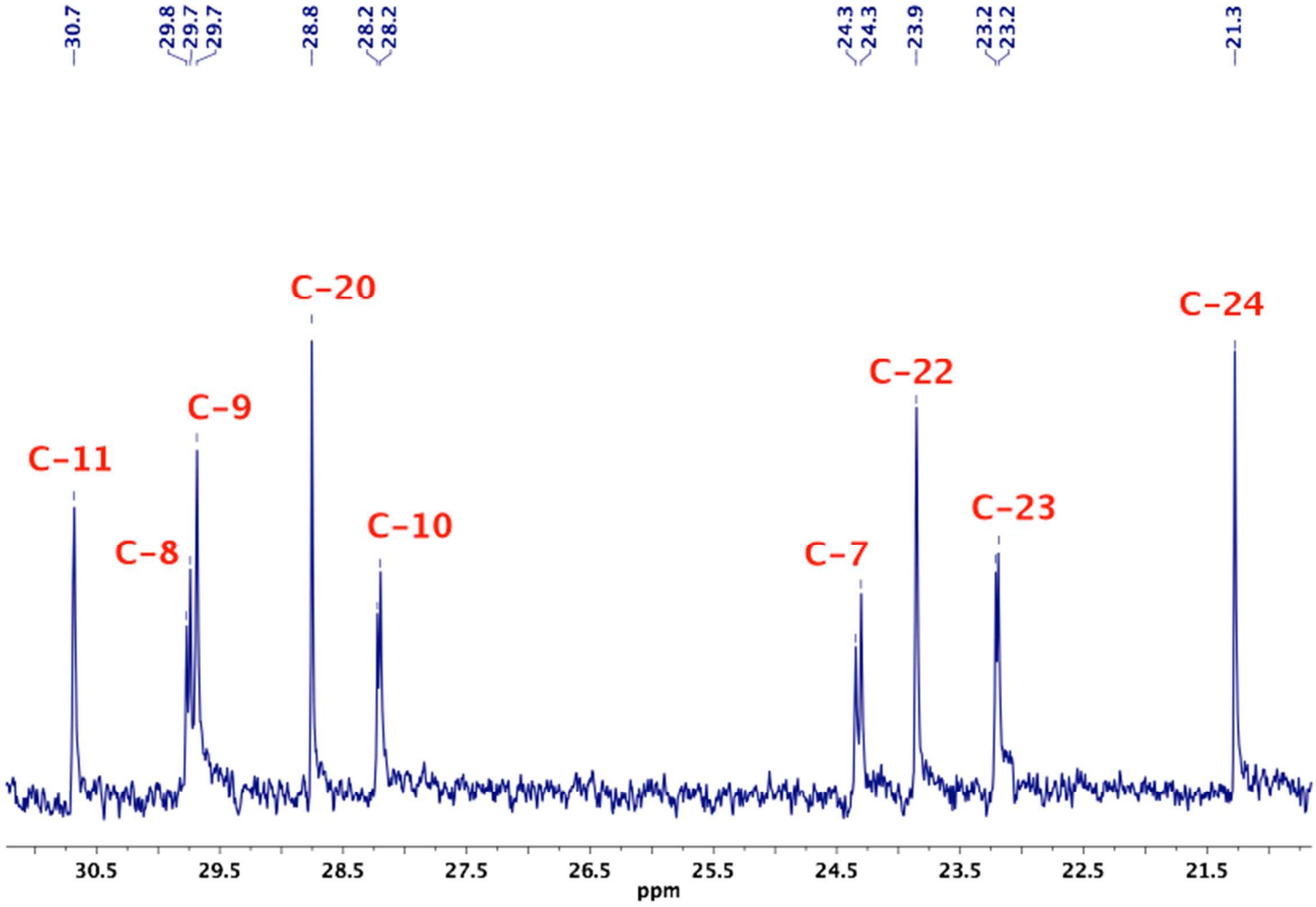
Note: Interestingly, in benzene-d_6 the signals ascribable to H_3-22 and H_3-23 of the 1,2-dioxolane array appear in pairs, whereas those for H_3-24 and H_3-21 (assigned to the bicyclo[4.2.0]octene system) do not split.

Expansion of the ^{13}C-NMR spectrum (125 MHz) of the mixture of plakortinic acids A (**2**) and B (**3**) in $CDCl_3$.



Note: Upon expansion, some of the ^{13}C-NMR resonances appear as narrowly split pairs.

Expansion of the ^{13}C-NMR spectrum (125 MHz) of the mixture of plakortinic acids A (**2**) and B (**3**) in $CDCl_3$.



Note: In this expansion, only the ^{13}C-NMR resonances assigned to C-7, C-8, C-10, and C-23 are observed as pairs.

Underwater photograph of the sponge consortium *Plakortis halichondrioides–Xestospongia deweerdtae*.