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Validating an Endoperoxide as a Key Intermediate in the Biosynthesis of Elysiapyrones

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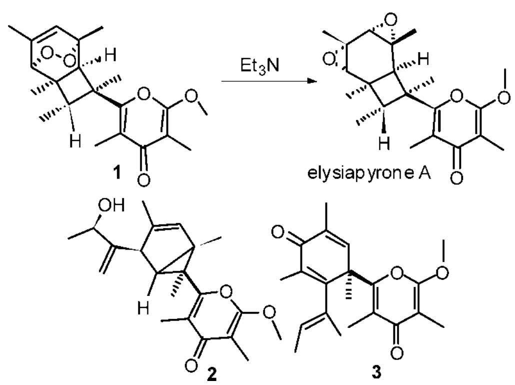
ABSTRACT



Compounds 1−3 isolated from *Elysia diomedea* are described. Compound 1 is an endoperoxide derivative of elysiapyrone A. The biomimetic-type transformation of compound 1 to elysiapyrone A catalyzed by neutral base transformed the endoperoxide to a vicinal diepoxide, thus suggesting the endoperoxide as a key intermediate in the biosynthesis of elysiapyrone A. A biogenetic pathway for their formation involving a cycloaddition of singlet oxygen to a polypropionate alkenyl open chain is proposed.

The sacoglossan *Elysia* (= *Tridachiella*) *diomedea*'s (Bergh) (Mollusca, Opisthobranchia, Sacoglossa) lack of a protective shell and chemical defense mechanisms against predators supports its survival strategies.[1] Diet-derived and, mostly, endogenous feeding deterrents are biosynthesized by joining several propionate units, two of which are involved in the formation of an α-methoxy-γ-pyrone ring that has become a common feature of the Elysiidae family. These sea slugs retain chloroplasts harvested from siphonous marine algae which enable them to live autotrophically.[2]

Polypropionated-derived polycyclic metabolites from this genus evolve from achiral conjugated polyene precursors which are prone to undergo isomerizations and cyclizations resulting in complex ring systems. For example, the related pairs, 15-nor-9,10-deoxytridachione/15-norphotodeoxytridachione, 9,10-deoxytridachione/photodeoxytridachione, and elysione/crispatene[3] (Figure 1), are suspected to arise by means of photochemical reactions.[4] This isomerization has been proven biosynthetically in vivo as well as in vitro

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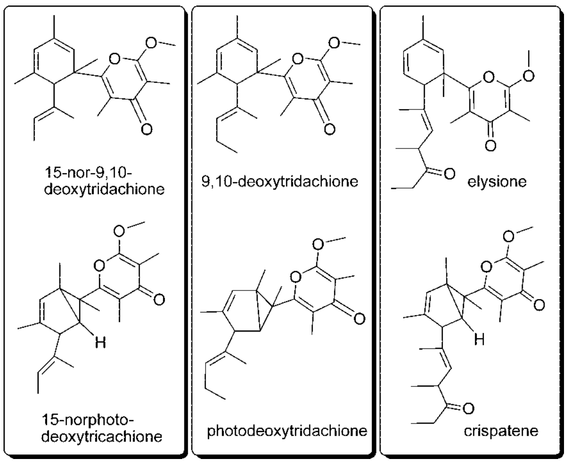

Figure 1. Pairs of cyclohexadiene/bicyclo[3.1.0]hexene photoproducts.

demonstrating that the bicyclo[3.1.0] core evolves from the cyclohexadiene ring. Since retention of optical rotation was observed during the process, it was proposed to proceed through a concerted $[_{\sigma}2_a + _{\pi}2_a]$ isomerization.[5] Nevertheless, it has been suggested that the bicyclic core may also arise from its corresponding acyclic precursor either by thermal $[_{\pi}4_a + _{\pi}2_a]$ cycloaddition[6] or intramolecular photochemical $[_{\pi}4_s + _{\pi}2_a]$ Diels−Alder reaction.[7]

Compound **1**, along with the novel compounds **2** and **3** (Figure 2), and the known metabolites tridachione, 9,10-deoxytridachione,[8] 15-norphotodeoxytridachione,[9] iso-9,10-deoxytridachione,[10] and elysiapyrones A and B[3] were isolated from the study of *Elysia diomedea* collected in the Gulf of Panama. Compound **2**, related to 15-norphotodeoxytridachione, contains a hydroxylic side chain, and compound **3** is a nor-derivative of tridachione.[8] The discovery of the naturally occurring polypropionate-derived endoperoxide **1** and its biomimetic transformation into the diepoxide elysiapyrone A contribute to support our proposal of a biogenetic pathway for elysiapyrone A in *Elysia diomedea*.

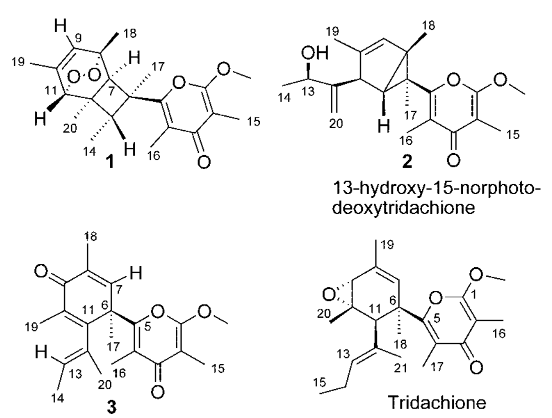

Figure 2. Novel metabolites **1**−**3** from *Elysia diomedea*.

Compound **1** was isolated as a colorless oil, $[\alpha]_D^{25} = +275$ (c 0.08, $CHCl_3$). The molecular formula suggested by the HREIMS, $[M]^+$ 360.1935 (calcd for $C_{21}H_{28}O_5$, 360.1936), indicates eight degrees of unsaturation. The ^{13}C NMR data showed signals for 21 carbons, and DEPT spectral data indicated the presence of eight methyl groups, four methine carbons (one bearing oxygen and another olefinic), five quaternary olefinic carbons, one carbonyl, and three sp^3 quaternary carbons. The ^1H NMR spectrum showed the following eight methyl group signals: one methoxy group (δ 3.90), three olefinic methyls (δ 1.85, δ 1.94, and δ 2.01), three methyls on quaternary carbon (δ 1.21, δ 1.48, and δ 1.49), and a secondary methyl group (δ 0.98 d, $J = 7.4$ Hz). Additional signals at δ 2.89 (q, $J = 7.4$ Hz) for a methine quartet (COSY coupled with a secondary methyl group) as well as three methines (one bearing oxygen at δ 3.94 and another olefinic at δ 5.64 s) complete all the protons of **1**.

All C−H correlations were detected in the HSQC spectrum. Compound **1** has an identical molecular formula to and exact mass as elysiapyrone A.[3] The difference between both compounds just lies in the substitution pattern of the heteroatoms attached to the six-membered ring of the bicyclo[4.2.0]octane core. Whereas elysiapyrone A contains two oxygen atoms in the form of a diepoxide on a cyclohexane ring, in **1** these oxygens must be in the form of an endoperoxide since the six-membered ring of the bicyclo[4.2.0]octane core contains a double bond. This was corroborated by the long-range correlations of H-9 with both C-18 and C-19; H-11 with C-19 and C-20; as well as H-7 with C-18 and C-20. Therefore, the planar structure of endoperoxide **1** is as depicted in Figure 2.

13-Hydroxy-15-norphotodeoxytridachione **2** was isolated as a colorless oil, $[\alpha]_D^{25} = +100$ (c 0.12, $CHCl_3$). The molecular formula suggested by the HREIMS, 344.1986 $[M]^+$ (calcd for $C_{21}H_{28}O_4$ 344.1988), indicates eight degrees of unsaturation. Absorptions for a carbonyl and a hydroxyl group at 1542 and 3471 cm^{-1}, respectively, were observed in the IR spectrum. The NMR spectral data of **2** resemble those of the 15-norphotodeoxytridachione,[9] also isolated in this work, except those corresponding to the side chain. Since **2** has the same number of unsaturations as 15-norphotodeoxytridachione but 16 u more, the side chain must be hydroxylated. This was corroborated by the HMBC correlations of H-13 with C-14 and C-20. Thus, **2** is a hydroxyl derivative of 15-norphotodeoxytridachione.

Nortridachidione **3** was isolated as a colorless oil, $[\alpha]_D^{25} = -50$ (c 0.24, $CHCl_3$). The molecular formula suggested by the HREIMS, 342.1825 $[M]^+$ (calcd for $C_{21}H_{26}O_4$

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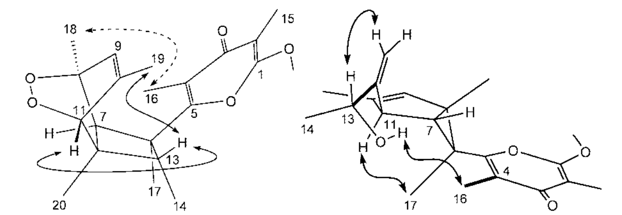
342.1831), indicates nine degrees of unsaturation. Absorption for unsaturated carbonyl groups at 1547 cm^{-1} was observed in the IR spectrum. The NMR data of the γ-pyrone unit of nortridachidione are almost coincident with those of tridachione, also isolated in this work. The main differences between both compounds lie on the substitution pattern of the carboxycyclic rings. The long-range HMBC correlations of H_3-18 with C-7, C-8, and C-9 and the correlations of H_3-19 with C-9, C-10, and C-11 indicate this half of the molecule contains a substituted 1,6-dimethyl cyclohexadienone system. HMBC correlations of H_3-20 and H-13 with C-11 allowed us to link a 2-butene fragment to the cyclohexadienone ring. Both the γ-pyrone and the substituted cyclohexadienone fragment were linked through C-5−C-6 due to the long-range correlations of H-7 and H_3-17 with C-5. Thus, the planar structure of **3** resulted to be a nor-derivative of tridachione,[8] and it was named nortridachidione. The (*E*)-geometry of the C-12−C-13 double bond was determined from the ^{13}C chemical shift of C-20 (δ 16.9 ppm). If the geometry of the double bond was *Z*, a downfield shift of about 5 ppm should be expected for that carbon due to a γ effect in a (*Z*)-relationship.[11]

NOESY experiments (Figure 3) aided to establish the stereochemistry of the substituents on the bicyclo[4.2.0]octane core of **1** as well as the configuration at C-13 of **2**. NOESY experiments of **1** revealed a *syn*-periplanar relationship between three vicinal angular methyl groups (Me-14, Me-17, and Me-20); thereby, the six-membered rings are face-to-face on the same side of the cyclobutane ring. Additional NOEs, particularly H-13/H-11 and H-13/H_3-19, fixed the stereochemistry of the endoperoxide functionality. Molecular mechanics energy minimizations of **1** and **2** were performed[12] (Figure 3). The minimized structure **1** led to interatomic distances appropriate for the NOEs observed. Thus, the overall relative stereochemistry of compound **1** is: 6*S, 7*R, 8*S, 11*R, 12*R, and 13*R. NOESY experiments of **2** showed an NOE between H-11 and H_3-17 which established the relative configuration of the bicyclo[3.1.0]hexane. Also, strong NOEs between H-13 and one proton of the olefinic methylene H_2-20 as well as between the proton of the hydroxyl group with Me-16 were observed. As the interatomic distance for the latter calculated by the program in the minimized structure of **2** justified the observed NOE, we proposed an *R relative configuration for C-13, being the overall stereochemistry of **2**: 6*S, 7*R, 8*S, 11*R, 13*R.



1 **2**

Figure 3. Selected NOEs for compounds **1** and **2**.

It is well-known that thermolysis, photolysis, and metal ion-mediated rearrangements of unsaturated bicyclic endoperoxides to form alkoxy radicals are commonly used transformations to obtain *syn*-diepoxides and β,γ-epoxyketones.[13–15] However, the actual transformation mechanism to form diepoxide and epoxyketone is still unclear.[16]

It was unexpected that endoperoxide **1** undergoes rearrangement to a vicinal *syn*-diepoxide yielding elysiapyrone A as the sole product of reaction when treated with triethyl amine at room temperature (Figure 4). Since neither the

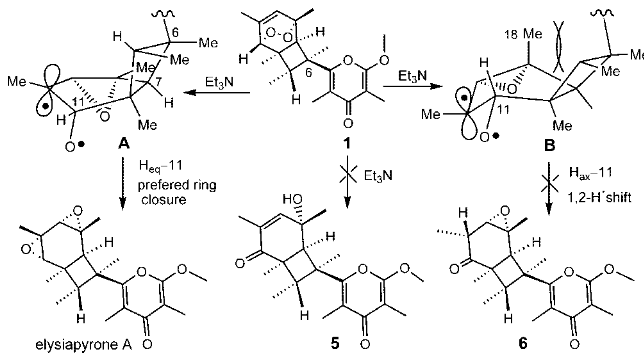


Figure 4. Biomimetic conversion of endoperoxide **1** to elysiapyrone A.

expected base-catalyzed Kornblum DeLaMare[17] β-elimination product, the hydroxyketone **5**, nor the epoxyketone **6** were observed, the reaction is not a base-catalyzed E2-elimination. The role of triethylamine in the reaction mechanism is unclear, but the exclusive formation of **1** could be explained assuming that the diepoxide is formed from a biradical intermediate. Conformational factors may favor an equatorial C−H bond in the biradical intermediate A, allowing ring closure to elysiapyrone A. A transition state like B in which a parallel arrangement of a p-orbital on carbon with the adjacent axial C−H bond would facilitate the 1,2-hydrogen shift required to yield epoxyketone seems unfavorable due to steric interaction between the axial Me-18 and the γ-pyrone ring.

We have recently reported the isolation of two complex polyketide derivatives elysiapyrone A and elysiapyrone B containing a bicyclo[4.2.0]octane core that features a novel carbon skeleton, elysiapyrone.[3] Since endriandric acid[18] was reported, several new biomedical important metabolites

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sharing this unusual architecture have been found.[19,20] These compounds may arise from a pathway (pathway a, Figure 5) involving a tandem thermal 8π conrotatory followed by a 6π disrotatory electrocyclization of an achiral polyene precursor resembling Black's hypothesis for the formation of endriandic acids.[21]

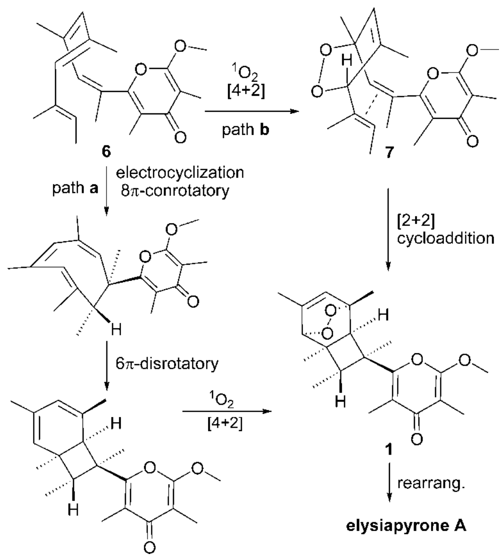

Figure 5. Proposed biogenesis for compound **1** and elysiapyrone A.

So far, there is no description on the specific conditions by which a substituted tetraene like **6** is naturally transformed into elysiapyrone A. In this study, we propose as a likely mechanism an intramolecular [2 + 2]-cycloaddition of the endoperoxide **7** generated by [4 + 2]-cycloaddition of singlet oxygen to the corresponding tetraene **6**. In fact, a biomimetic synthesis of racemic elysiapyrones A and B that involves an endoperoxide as a key step to generate the diepoxide functionality has been successfully reported by Trauner et al.[22] In Trauner's strategy, the isomerization endoperoxide→diepoxide was achieved by using transition metal catalysis. The ^1H NMR spectrum of the synthetic endoperoxide displays signals that exactly reproduce those obtained for the natural product (see Supporting Information).

Our endeavor to mimic the natural process to achieve the transformation of **1** suggested that the endoperoxide is a key intermediate in the biosynthesis of elysiapyrone A. Other naturally occurring endoperoxides may also follow a similar biogenetic pathway.[23,24] Moreover, trapping methods with chemicals such as β-carotene that specifically react with singlet oxygen succeeded in isolating and identifying β-carotene 5,8-endoperoxide and β-carotene 5,6-epoxide ex vivo and in vivo systems[25] support this hypothesis. Because both compound **1** and elysiapyrone A are optically active metabolites, their biosynthesis must be enzymatically assisted.

Since toxic levels of reactive oxygen species (ROS) can damage the photosystem (PS) protein-dependent chloroplast activity,[26] the presence of the endoperoxide **1** may alleviate the symbiotic plastids from light-induced damage, conferring advantages in adaptive responses of *E. diomedea*.

Acknowledgment. This work was supported by the Ministerio de Educación y Ciencia (BIO2007-61745, SAF2006-03004) and DGUI Gobierno de Canarias (PIO42005, PUB2005/030). The STRI provided support and facilities for field work. Dr. J. Maté and J. del Rosario provided technical support. The Government of Panama granted permission for the collection of the samples.

Supporting Information Available: Spectral data and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information

Validating an endoperoxide as a key intermediate in the biosynthesis of elysiapyrones

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1-3** [500 MHz, δ ppm, (*J*) Hz, $CDCl_3$]

	1		**2**		**3**	
#	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	---	161.6	---	162.5	---	161.9
2	---	99.6	---	99.5	---	99.9
3	---	181.3	---	181.6	---	180.6
4	---	118.5	---	120.4	---	121.8
5	---	159.7	---	160.1	---	153.6
6	---	45.0	---	41.2	---	48.3
7	2.20 (s)	58.2	1.24 (s)	31.6	6.62 (s)	146.5
8	---	77.0	---	32.7	---	133.3
9	5.64 (br s)	126.2	5.42 (s)	129.6	---	186.4
10	---	142.8	---	144.0	---	131.9
11	3.94 (d, 2.1)	82.9	2.90 (s)	50.2	---	157.7
12	---	36.8	---	153.2		132.6
13	2.89 (q, 7.4)	35.8	4.37 (q, 6.4)	70.8	5.07 (m)	124.8
14	0.98 (d, 7.4)	10.5	1.37 (d, 6.4)	22.2	1.61 (d)	13.6
15	1.85 (s)	6.9	1.86 (s)	6.8	1.85 (s)	6.9
16	1.94 (s)	12.6	1.99 (s)	10.8	1.72 (s)	9.1
17	1.48 (s)	23.5	1.15 (s)	13.1	1.61 (s)	24.2
18	1.21 (s)	21.7	1.19 (s)	17.3	1.95 (s)	15.8
19	2.01 (d, 1.4)	20.6	1.64 (s)	13.8	1.83 (s)	12.7
20	1.49 (s)	19.0	4.84 (s) 5.20 (s)	112.1	1.60 (s)	16.9
21	3.90 (s)	55.3	3.95 (s)	55.2	3.93 (s)	55.5
O*H*			1.53 (s)			

Experimental

General procedures

Optical rotations were measured on a Perkin-Elmer model 343 Plus polarimeter using a Na lamp at 25 ºC. IR spectra were obtained with a Perkin-Elmer 1650/FTIR spectrometer. ^{1}H NMR and ^{13}C NMR, HSQC, HMBC and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Two-dimensional NMR spectra were obtained with the standard Bruker software. LREIMS and HREIMS data were taken on a Micromass Autospec spectrometer. HPLC separations were performed with a Hewlett Packard 1050 (Jaigel-Sil semipreparative column, 10μ, 20x250 mm) eluted with hexane-EtOAc mixtures. The gel filtration column (Sephadex LH-20) used hexane-MeOH-CH_2Cl_2 (3:1:1) as solvent. The spray reagent for TLC was H_2SO_4-H_2O-AcOH (1:4:20).

Biological Material

One hundred and twenty specimens of *Elysia diomedea* were collected by hand off Saboga Island (Panama) at –1.5 m.

Extraction and Isolation

Wet samples were extracted with methanol at room temperature, and were concentrated to give a dark residue (5.0 g). The extract was partitioned between EtOAc (3x75 ml) and water (75 ml). The EtOAc extracts were combined to obtain a brown oil (643.5 mg) that was chromatographed on a LH-20 column. Fractions containing pyrones, as indicated by their ^{1}H NMR spectra, were further chromatographed on HPLC to give compounds **1** (0.8 mg, 0.016%), **2** (1.2 mg, 0.024%) and **3** (2.4 mg, 0.048%) and the known compounds tridachione (77.7 mg, 1.554%), 9,10-deoxytridachione (15.2 mg, 0.304%), 15-norphotodeoxytridachione (3.2 mg, 0.064%), and iso-9,10-deoxytridachione (2.2 mg, 0.044%).

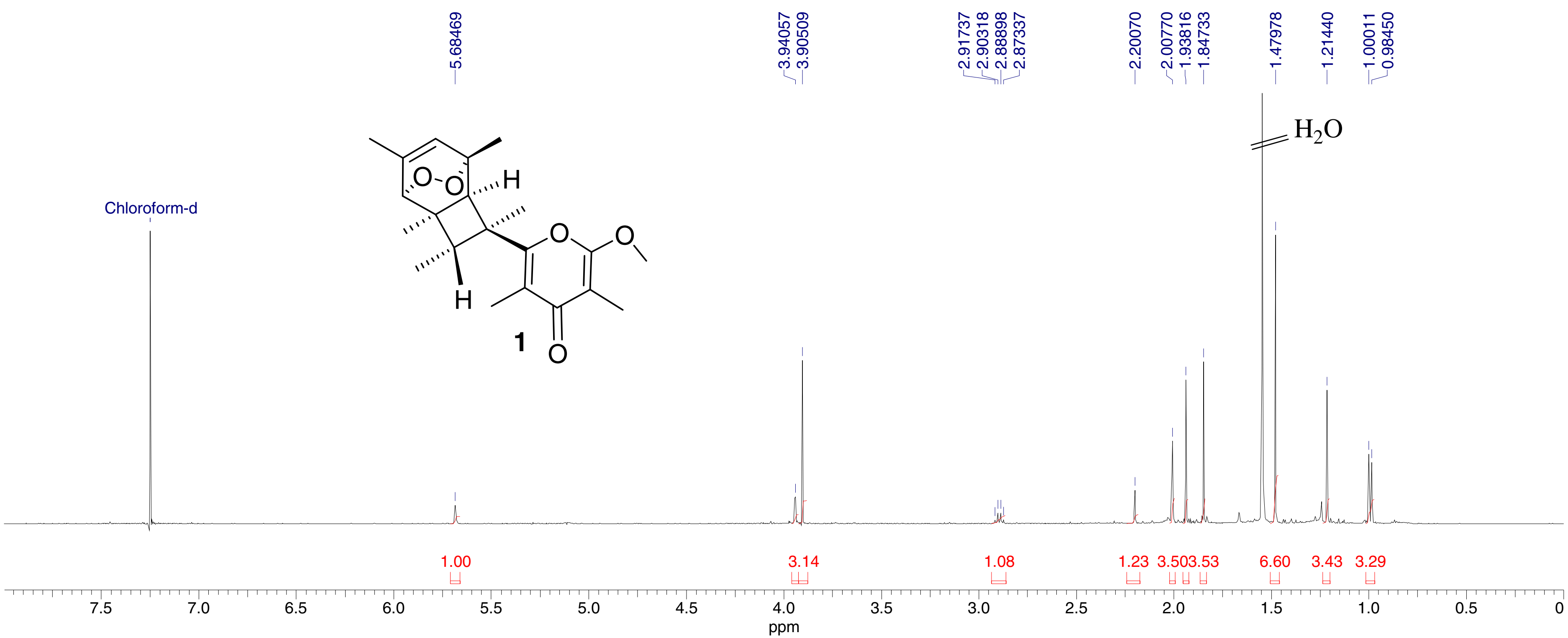
Compound (1). Colorless oil; $[\alpha]^{25}_{D}$ +275 (*c* 0.08, $CHCl_3$); IR ν_{max} 1548 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; LREIMS *m/z* 360 $[M]^+$ (12), 345 $[M - Me]^+$ (4), 326 (5), 317 $[M - Me - CO]^+$ (8), 209 (99), 193 (100); HREIMS $[M]^+$ 360.1935 (calcd for $C_{21}H_{28}O_5$, 360.1936), $[M - Me]^+$ 345.1689 (calcd for $C_{20}H_{25}O_5$, 345.17019) $[M - Me - CO]^+$, 317.1763 (calcd for $C_{19}H_{25}O_4$, 317.1753).

Compound 2. Colorless oil; $[\alpha]_D^{25}$ = +100º (*c* 0.12, $CHCl_3$); IR (film): ν_{max} 3471, 1542 cm^{-1}; LREIMS (70 eV): *m/z* (%) 344 $[M]^+$ (14), 326 $[M - H_2O]^+$ (54), 311 (43), 197

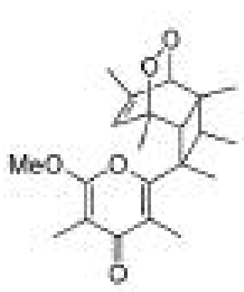
(88), 182 (90), 91 (100); HREIMS $[M]^+$ 344.1986 (calcd for $C_{21}H_{28}O_4$ 344.1988), $[M - H_2O]^+$ 326.1871 (calcd for $C_{21}H_{26}O_3$ 326.1882).

Compound 3. Colorless oil; $[\alpha]_D^{25}$ = +50º (*c* 0.24, $CHCl_3$); IR (film): ν_{max} 1547 cm^{-1}; LREIMS (70 eV): *m/z* (%) 342 $[M]^+$ (65), 327 $[M - Me]^+$ (85), 314 $[M - CO]^+$ (77), 299 $[M - Me - CO]^+$ (48), 213 (100); HREIMS $[M]^+$ 342.1825 (calcd for $C_{21}H_{26}O_4$ 342.1831), $[M - Me]^+$ 327.1596 (calcd for $C_{20}H_{23}O_4$ 327.1596), $[M - CO]^+$ 314.1889 (calcd for $C_{20}H_{26}O_3$ 314.1882).

^{1}H NMR of compound **1** in $CDCl_3$



[1]H NMR of compound **1** in $CDCl_3$ from supporting information of Barbarow, J. E.; Miller, A. K.; Trauner, D. *Org Lett.* **2005**, *7*, 2901-2903.



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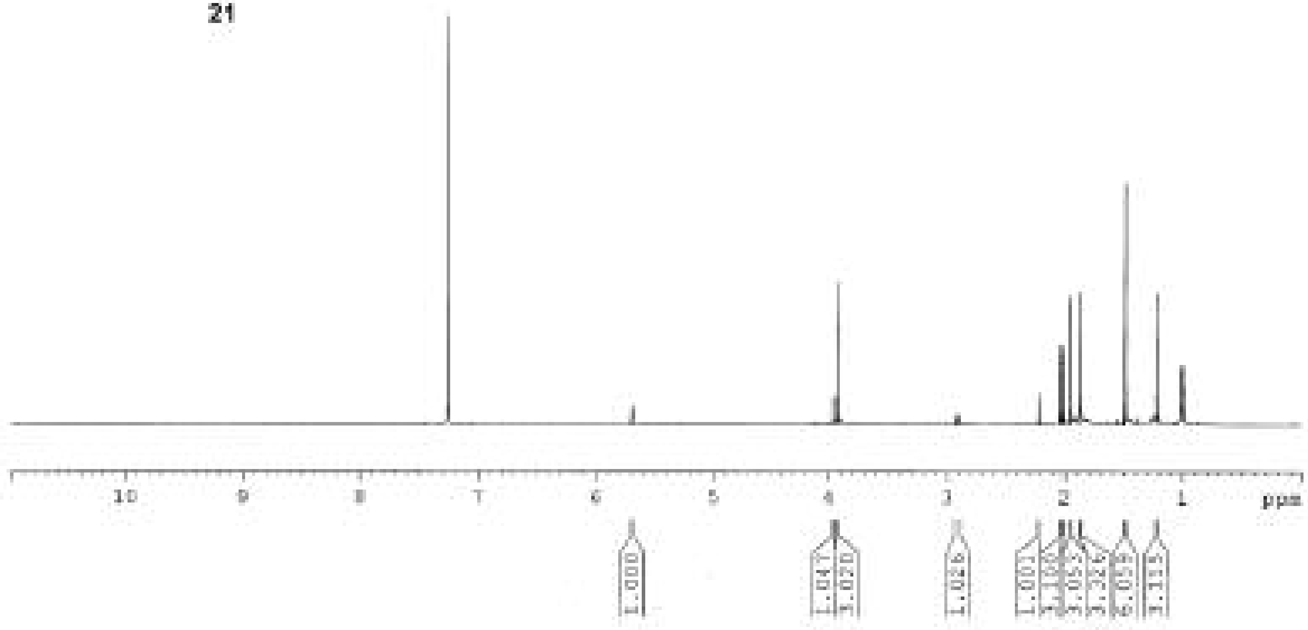
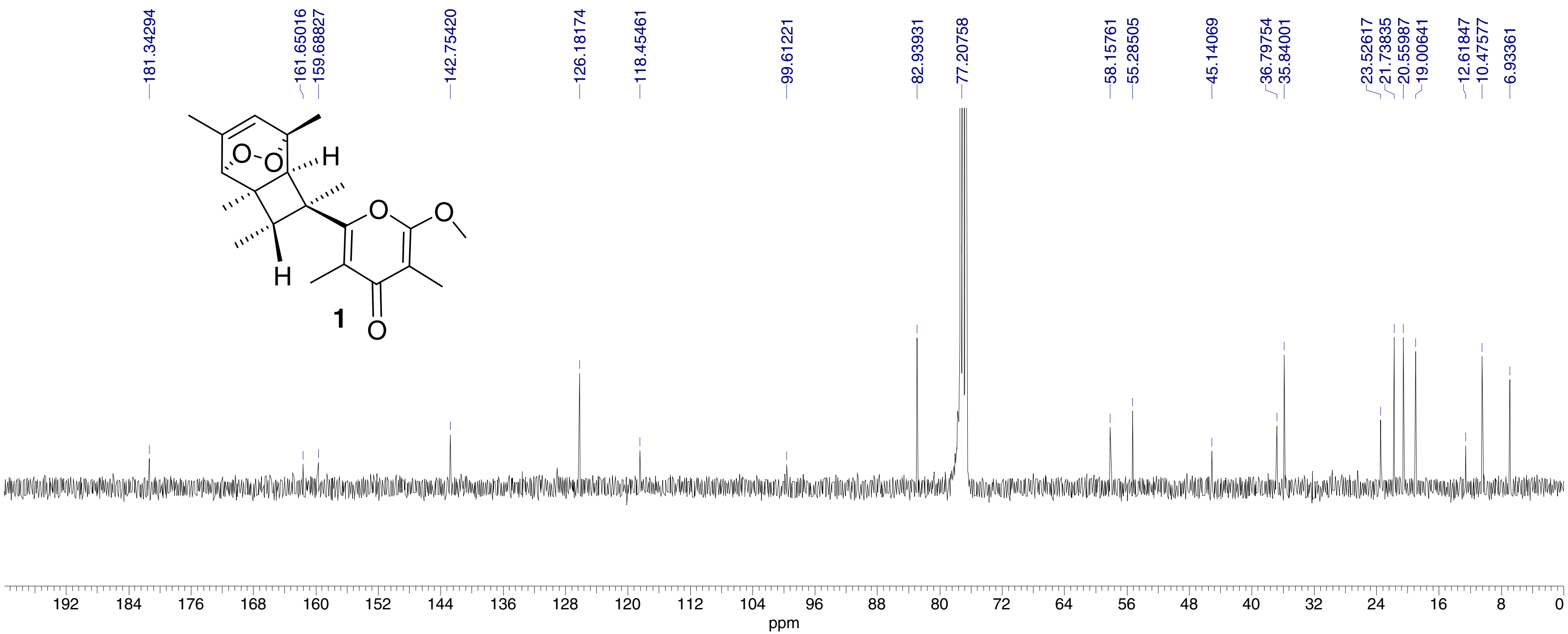
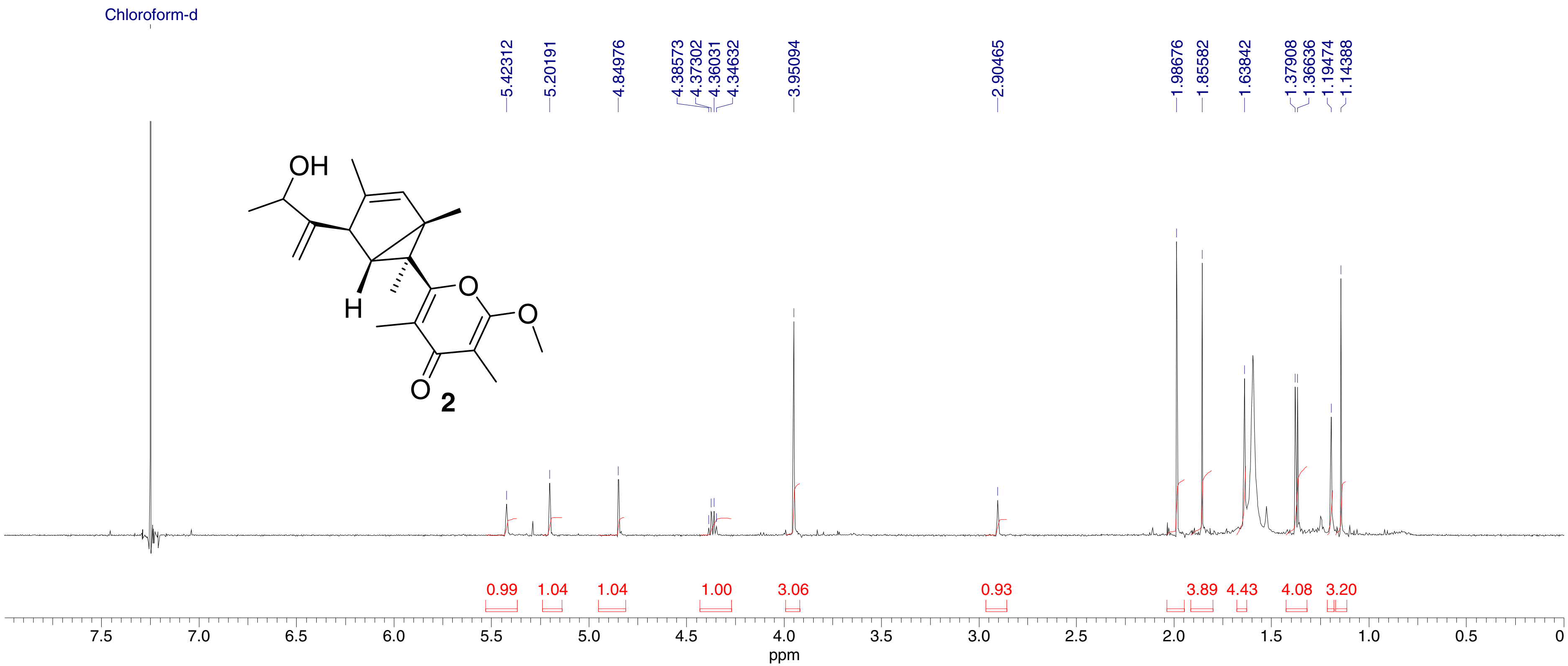


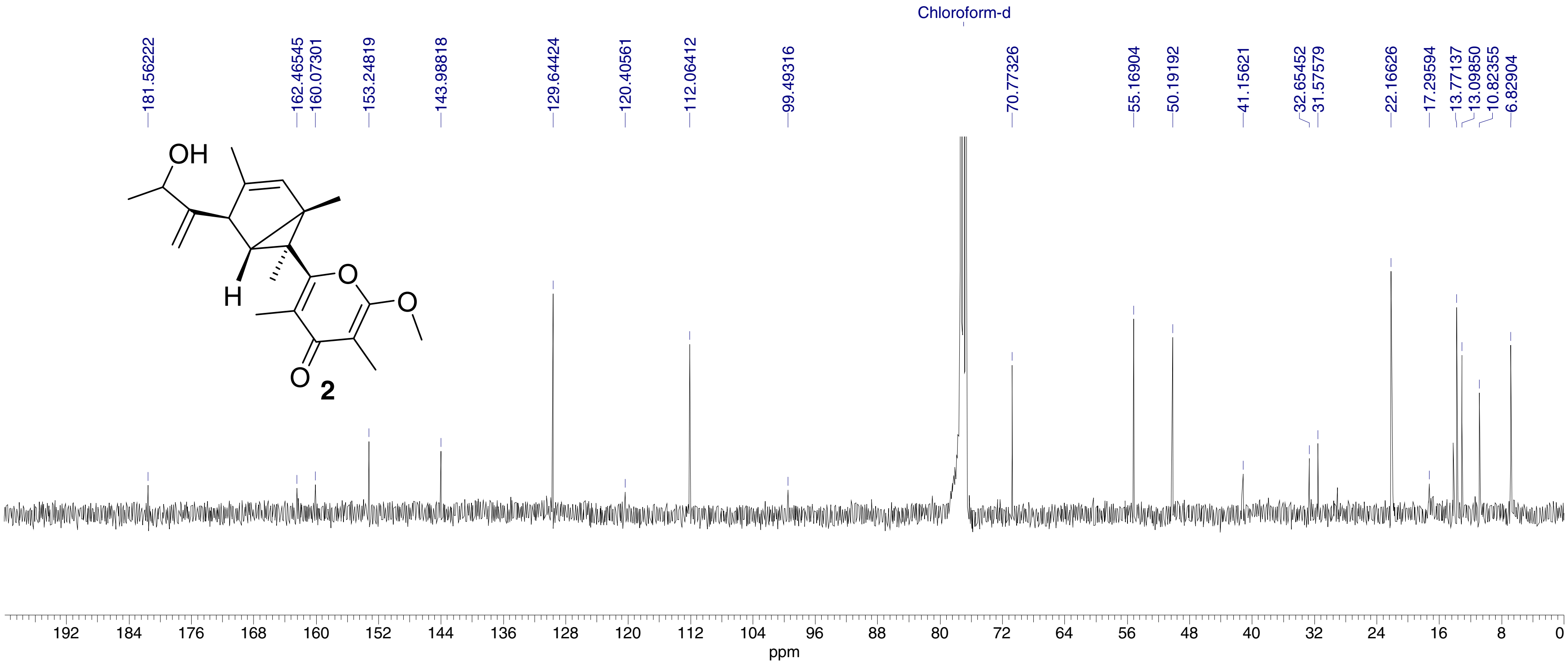
^{13}C NMR of compound **1** in $CDCl_3$



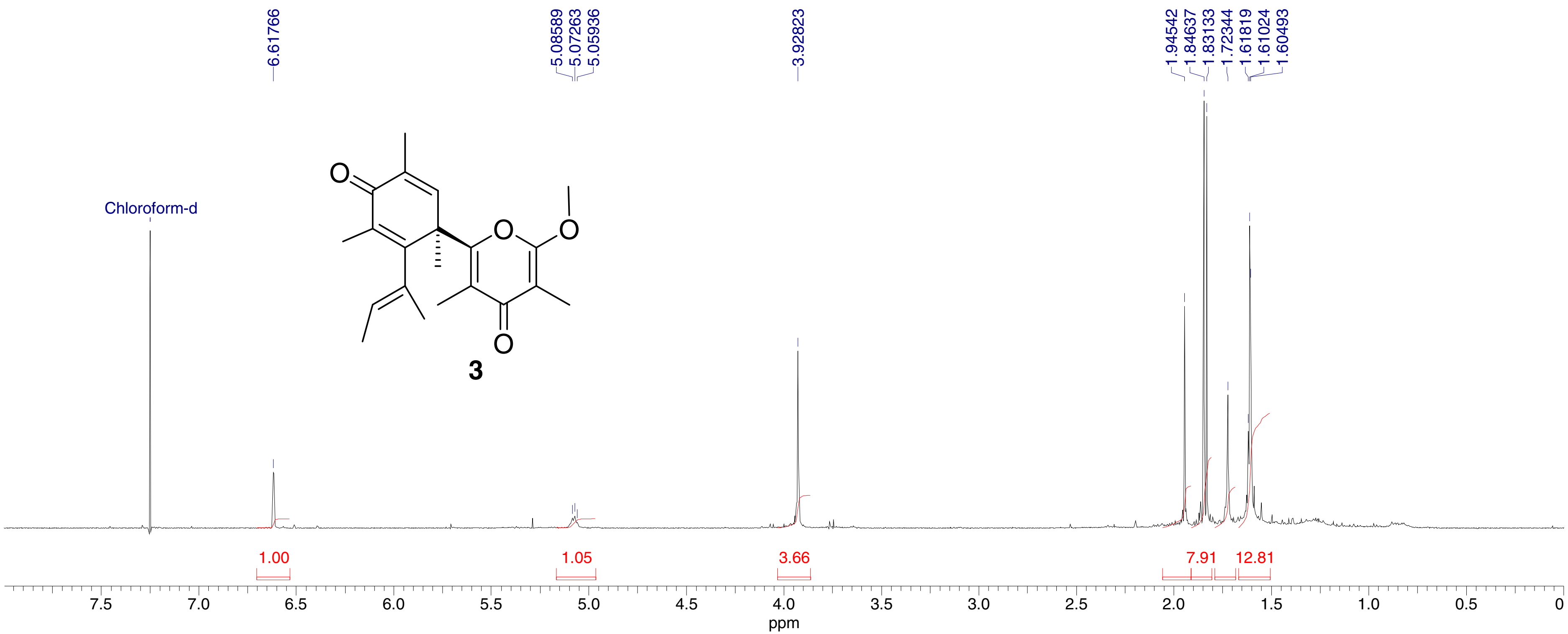
^{1}H NMR of compound **2** in $CDCl_3$



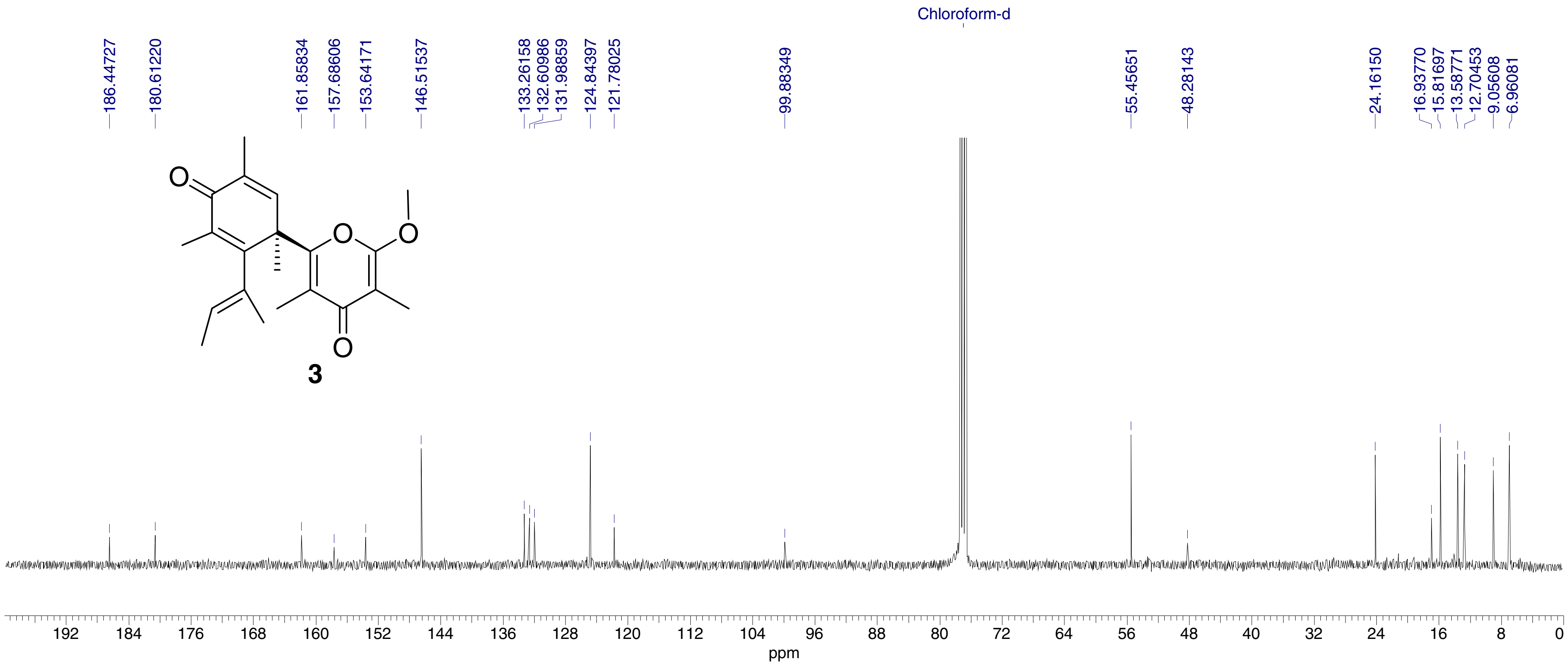
^{13}C NMR of compound **2** in $CDCl_3$

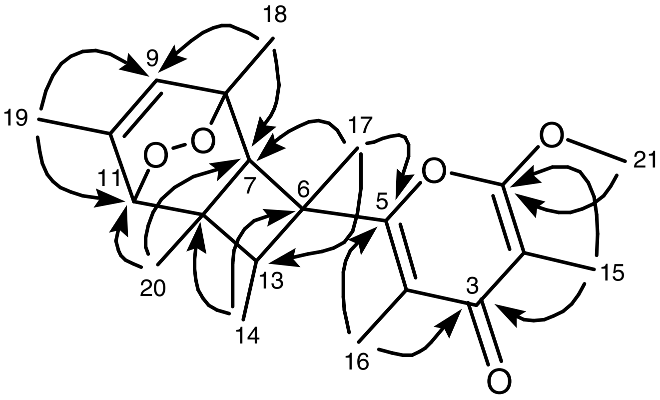


^{1}H NMR of compound **3** in $CDCl_3$



^{13}C NMR of compound **3** in $CDCl_3$





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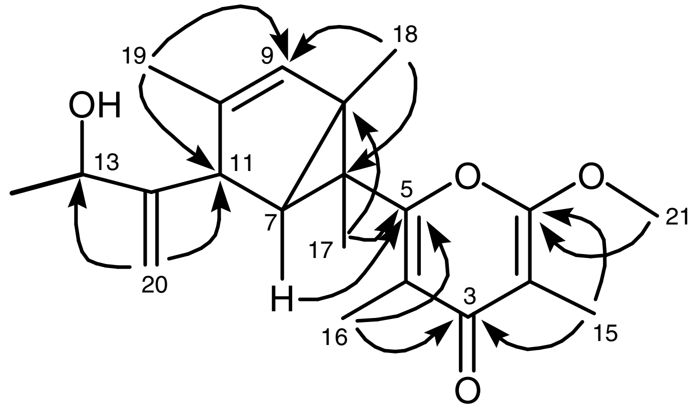
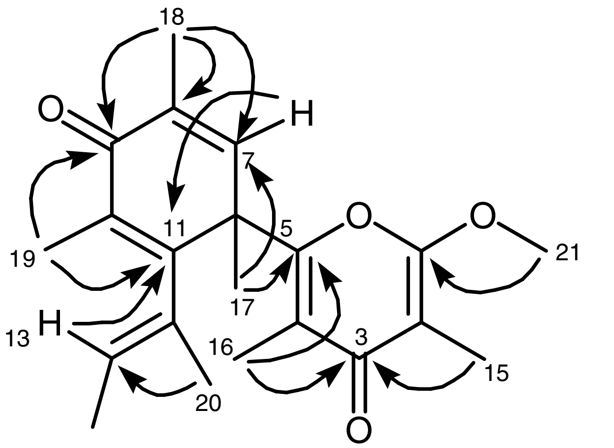


2



3

HMBC key correlations of compounds **1**-**3**

File:H7027 Ident:27 Acq:25-MAR-2003 04:28:22 +1:24 Cal:H7027
AutoSpec EI+ Magnet BpM:84 BpI:133888 TIC:1110593 Flags:ACC
File Text:Mercedes (P-5) E/I 70 ev Temp.F. 230C COMPOUND 1
Heteroatom Max: 20 Ion: Both Even and Odd
Limits:

Mass	%RA	Pks	Std	PPM	mDa	Calc. Mass	DBE	C	H	O
150.000	0.2						-0.5	0	0	0
430.000	100.0				10.0		20.0	30	40	5
361.190575	0.2			14.0	5.1	361.195626	16.5	28	25	
360.193558	1.0			0.3	0.1	360.193674	8.0	21	28	5
				-16.0	-5.8	360.187801	17.0	28	24	
345.168938	0.4			3.7	1.3	345.170199	8.5	20	25	5
				-13.4	-4.6	345.164326	17.5	27	21	
342.183022	0.3			0.3	0.1	342.183110	9.0	21	26	4
327.186264	0.2			29.8	9.8	327.196020	8.5	21	27	3
326.175949	0.6			-27.2	-8.9	326.167066	14.0	24	22	1
317.176270	0.6			-3.1	-1.0	317.175285	7.5	19	25	4
311.160110	0.3			14.8	4.6	311.164720	9.5	20	23	3
261.146042	0.9			11.6	3.0	261.149070	6.5	16	21	3
257.147324	0.4			26.6	6.8	257.154155	7.5	17	21	2
				-32.8	-8.4	257.138899	3.5	13	21	5

HRESIMS of compound **1**

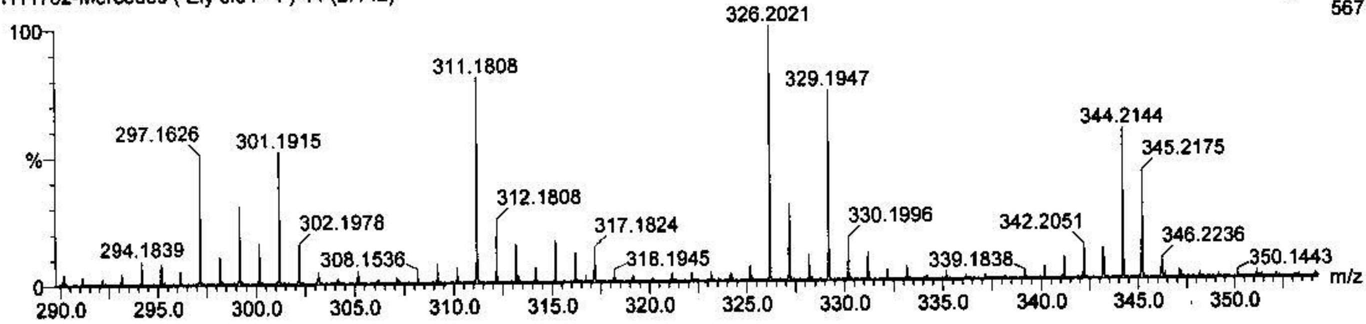
Elemental Composition Report

Multiple Mass Analysis: 88 mass(es) processed - displaying only valid results
Tolerance = 5.0 PPM / DBE: min = 1.5, max = 50.0
Isotope matching not enabled

Monoisotopic Mass, Odd and Even Electron Ions
657 formula(e) evaluated with 8 results within limits (up to 50 closest results for each mass)

Mercedes (Ely sic P-4) E/I. 70 ev. Temp. F. 260°C COMPOUND 2
H11782-Mercedes (Ely sic P-4) 44 (2.142)

Magnet EI+
567



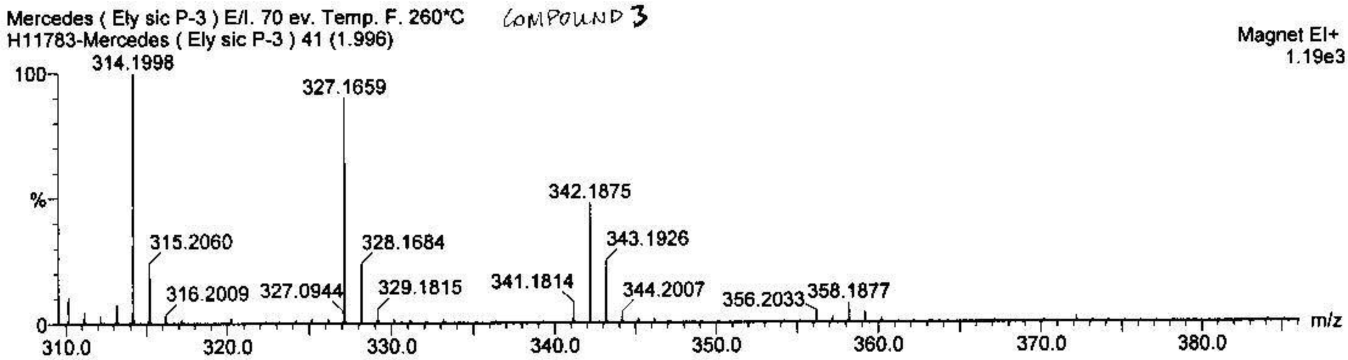
Minimum:	20.00				1.5	
Maximum:	100.00		200.0	5.0	50.0	
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula
344.1986	24.34	344.1988	-0.2	-0.5	8.0	C21 H28 O4
329.1758	26.10	329.1753	0.5	1.6	8.5	C20 H25 O4
326.1871	37.39	326.1882	-1.1	-3.4	9.0	C21 H26 O3
311.2023	26.98	311.2011	1.2	3.8	8.5	C21 H27 O2
311.1843	77.43	311.1858	-1.5	-5.0	4.5	C17 H27 O5
301.1812	29.45	301.1804	0.8	2.8	7.5	C19 H25 O3
299.1858	22.93	299.1858	0.0	-0.2	3.5	C16 H27 O5
297.1694	39.86	297.1702	-0.8	-2.7	4.5	C16 H25 O5

HRESIMS of compound **2**

Elemental Composition Report

Multiple Mass Analysis: 38 mass(es) processed - displaying only valid results
Tolerance = 5.0 PPM / DBE: min = 1.5, max = 50.0
Isotope matching not enabled

Monoisotopic Mass, Odd and Even Electron Ions
260 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)



Minimum:	20.00				1.5	
Maximum:	100.00		200.0	5.0	50.0	
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula
343.1926	24.66	343.1909	1.7	4.9	8.5	C21 H27 O4
342.1836	46.30	342.1831	0.5	1.4	9.0	C21 H26 O4
328.1684	23.57	328.1675	0.9	2.9	9.0	C20 H24 O4
327.1584	78.54	327.1596	-1.2	-3.8	9.5	C20 H23 O4
314.1889	71.38	314.1882	0.7	2.2	8.0	C20 H26 O3

HRESIMS of compound **3**